CONVENTIONAL PREPARATION VERSUS UPREP LIQUID BASED PREPARATION IN FINE NEEDLE ASPIRATION IN A TEACHING HOSPITAL – A COMPARATIVE STUDY

Dissertation submitted in partial fulfillment of the

requirements for the degree of

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"**CONVENTIONAL PREPARATION VERSUS UPREP LIQUID BASED PREPARATION IN FINE NEEDLE ASPIRATION IN A TEACHING HOSPITAL – A COMPARATIVE STUDY" is a bonafide work done by Dr. Aswathy Jayachandran**,** during the period 2015 –2018 under my direct supervision and guidance. This is submitted partial fulfillment of the requirement of The Tamilnadu Dr. M. G.R Medical University, Chennai for the award of M.D degree in Pathology.

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DECLARATION

I Dr. Aswathy Jayachandran hereby submit the dissertation titled "CONVENTIONAL PREPARATION VERSUS UPREP LIQUID BASED PREPARATION IN FINE NEEDLE ASPIRATION IN A TEACHING HOSPITAL – A COMPARATIVE STUDY" done in partial fulfillment for the award of the degree M.D Pathology in Sree Mookambika Institute Of Medical Sciences, Kulasekharam. This is an original work done by me under the guidance and supervision of Dr. Sivasankaran M. (MD).

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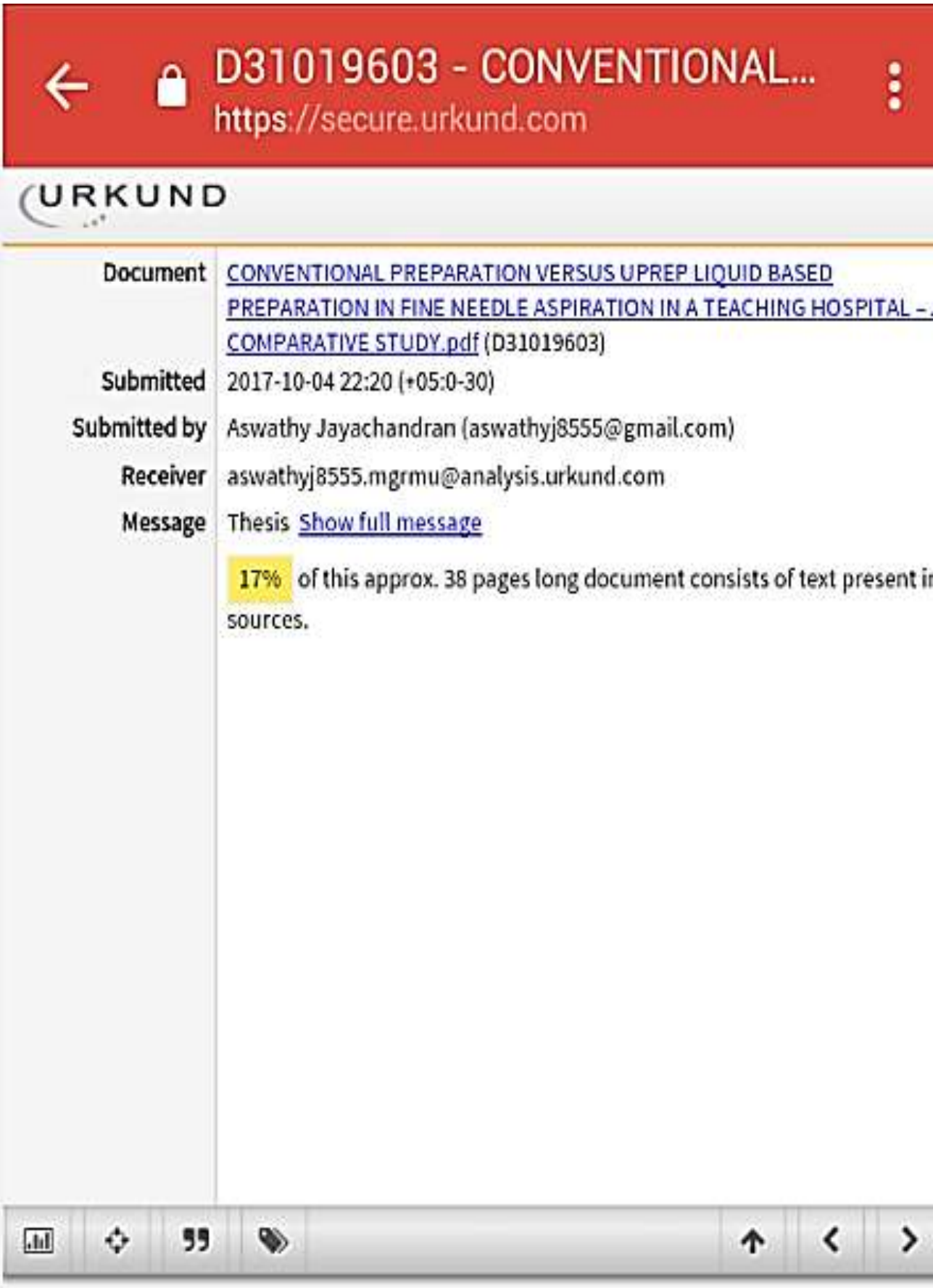
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CONTENTS

INTRODUCTION

Cancer is the leading cause of death worldwide, accounting for 8.8 million deaths in 2015 alone. Globally, nearly 1 in 6 deaths is due to cancer. Approximately 70% of cancer deaths occur in low- and middle-income countries. [1]

The number of new cancer cases is expected to rise by about 70% over the next 2 decades with more than 60% of world‟s total new annual cases occurring in Africa, Asia and Central and South America. These regions also account for 70% of the world‟s cancer deaths.

The most common causes for death worldwide are cancers of Lung (1.69 million deaths), Liver (788000 deaths), Colorectal (774000 deaths), Stomach (754000 deaths), Breast (571000 deaths). [1]

Carcinogenesis:

Cancers arise from the transformation of normal cells into tumour cells in a multistage process that encompasses the stepwise accumulation of multiple mutations that act in a complementary way. These changes are the result of the interaction between a person's genetic factors and 3 categories of external agents including:

- Physical carcinogens, like exposure to ionizing radiation and ultraviolet rays;
- Chemical carcinogens, like components of tobacco smoke, aflatoxin, asbestos and arsenic.
- Biological carcinogens, like infections from certain viruses, bacteria, or parasites.

Ageing is another fundamental factor for the development of cancer. The incidence of cancer rises dramatically with age, most likely due to a build-up of risks for specific cancers that increase with age. The overall risk accumulation is combined with less effective cellular repair mechanisms as a person grows older.

Between 30–50% of cancers can currently be prevented. This can be accomplished by avoiding risk factors and implementing the existing evidence-based prevention strategies. These risk factors include:

- Being overweight or obese.
- Unhealthy diet with low fruit and low vegetable intake.
- Lack of physical activity.
- Tobacco use, including cigarettes and smokeless tobacco.

- Alcohol abuse.
- Sexually transmitted HPV-infection.
- Infection by hepatitis or other carcinogenic infections.
- Ultraviolet and other ionizing radiation.
- Urban air pollution.
- Indoor smoke from household use of solid fuels.

Tobacco use is the single most important risk factor for cancer and is responsible for approximately 22% of cancer-related deaths globally. [(1)] The cancer burden can also be reduced through early detection of cancer and management of patients who develop cancer. Many cancers have a high chance of cure, if diagnosed early and treated adequately. Early diagnosis consists of 3 steps that must be integrated and provided in a timely manner.

1. Awareness and accessing care.
2. Clinical evaluation, diagnosis and staging.
3. Access to treatment.[(1)]

First reports of Fine needle aspiration cytology (FNAC) as a technique to obtain diagnostic material date back to the 19th century. FNAC was initially

a means to confirm a clinical suspicion of local recurrence or metastasis of known cancer without subjecting the patient to further surgical intervention. Following success in this area, the interest focused on preliminary preoperative diagnosis of all kinds of neoplastic processes, benign or malignant, in any organ or tissue of the body and on definitive, specific

diagnosis in inoperable cases as a guide to rational treatment. [63]

Fine Needle Aspiration Cytology (FNAC) when used alongside clinical and radiological assessment offers a relatively cheap, quick, and accurate tool for the diagnosis of cancer.[2] Due to its low cost, it is also being used for the differential diagnosis between benign and malignant lesions in the primary care units of many under developed countries.[9]

For many years, efforts have been made to develop methods to enhance the sensitivity and specificity of cytological smears. From this research and development, Liquid Based Preparation of cytological samples has evolved. Liquid-based cytology (LBC) is a technique that enables cells to be suspended in a monolayer, through which better morphological assessment is possible. It includes the preparation and evaluation of cells collected in a liquid fixative, the cells are then transferred in a representative manner and operator dependent variation will not occur. Though initially advised for gynaecology samples, it is increasingly being used for non-gynaecologic cytology samples and FNA samples also. [3, 4] Two technologies - Thin Prep (Cytyc Corp.) and SurePap (Tripath imaging, Inc.) have been more widely used and are FDA approved.[6]

Compared with conventional preparation, Liquid based Preparation has a number of advantages with regard to nuclear and cytoplasmic morphology, cell size and background material. Liquid based Preparation also allows for rapid fixation, decreased obscuring factors and standardisation of cell transfer. The advantages of liquid-based cytology include improved sensitivity and specificity, since fixation is better and nuclear details are well preserved. Abnormal cells are not obscured or diluted by other epithelial or inflammatory cells. There is, therefore, a lower rate of unsatisfactory cytology samples. [4, 8] The residual cell suspension can also be used to make further cytological preparations. More over immunocytochemistry can also be performed on the residual sample. [5]

Owing to the high cost of setup, manual methods of liquid based cytology are under evaluation. UPREP liquid based cytology system is a new and advanced system in the Manual Liquid based Preparation. The principle behind UPREP Liquid based Cytology procedure is surface adsorption by RCF [6].

AIMS AND OBJECTIVES:

To compare FNAC smears made by conventional preparation and UPREP liquid based preparation.

REVIEW OF LITERATURE

HISTORICAL ASPECTS:

Since the 15th century, syringes or equivalent instruments have been used to aspirate collections of fluids. With the introduction of achromatic microscopes and their industrial production in the 1830s, the microscope became cheaper and hence accessible to many observers who used it to examine the aspirated material. It has been mentioned that a French physician, Kün, and a German-Swiss pathologist, Lebert, described in 1847 and 1851, the use of a cannula to secure cell samples from palpable tumours and used the microscope to identify cancer. Sporadic use of aspirated samples has been described in the literature of the second half of the 19th century and in the first years of the 20th century. An important contribution was published in 1905 by two British military surgeons, Greig and Gray, working in Uganda who aspirated the swollen lymph nodes, by means of a needle and a syringe, in patients with sleeping sickness to identify the motile trypanosome.

By the beginning of the 20th century, the first aspiration biopsy aiding in diagnosis of a solid tumor was published by Hirschfeld (1912), who also became the first person to use a small-calibre needle. The most notable development in diagnostic aspiration biopsy was a paradoxical event. James Ewing, the Director of the Memorial Hospital for Cancer in New York City and Professor of Pathology at Cornell University Medical School, who was also a

dominant figure in American oncologic pathology between 1910 and 1940. Although Dr. Ewing has made great contributions to the classification and identification of human cancer, he adamantly opposed to tissue biopsies because he believe that it allegedly contributed to the spread of cancer. Due to the ban on tissue biopsies, Hayes Martin a young surgeon and radiotherapist at the Memorial Hospital, began to aspirate palpable tumours of various organs by means of a large-calibre needle and a Record syringe. His colleague Edward Ellis prepared the material in the form of air-dried smears and stained with haematoxylin and eosin. Tissue fragments (named clots) were further embedded in paraffin and processed as cell blocks. Palpable lesions of lymph nodes, breast, and thyroid were the initial targets of aspiration. The material was interpreted by Ewing's associate and subsequent successor Dr. Fred W. Stewart.

This method proved to be very successful and accurate with very few errors and clinical complications. Martin and Ellis published their conjoined initial results in 1930 and 1934. In 1933, Dr. Fred W. Stewart published a classic article, “The Diagnosis of Tumours by Aspiration,” in which he discussed, at length, the pros and cons of this method of diagnosis, its achievements, and pitfalls, based on experience with 2500 samples. This method of aspiration pioneered by Martin has remained a standard diagnostic procedure at Memorial Sloan-Kettering Cancer Center, the only institution in the world where the procedure has remained in constant use for more than 75 years.

In the 1940s, Paul Lopes-Cardozo and Nils Söderström, experimented on a large scale with this system of diagnosis, using small-calibre needles and hematologic techniques to process the smears.

By the 1970s, special aspiration biopsy clinics were established in Stockholm and Sweden to which patients with palpable lesions were provided referral to higher centres, for diagnosis. The technique became an acceptable substitute for tissue biopsies.

Broad acceptance of exfoliative cytology techniques (Pap smears) for detection and diagnosis of cervix cancer, played a major role in the development and acceptance of Fine needle aspiration.

After timid beginnings in the early 1970s, a new era of diagnosis began, pathologists started accepting the cytologic sample as clinically valid and important. By late 1900‟s biopsy by aspiration, also known as thin- or fine-needle aspiration biopsy (FNA), had become an important diagnostic technique, sometimes replacing but often complementing tissue pathology in many clinical situations.

In 1987 an article in the Wall Street Journal by an investigative journalist, Walt Bogdanich, on the failure of laboratories to identify cancer of the cervix in young women, elicited a great deal of attention. This prompted the Congress of the United States in 1988 to promulgate a law, known as the Amendment to the Clinical Laboratory Improvement Act (CLIA 88),

governing the practice of gynecologic cytology in the United States. Suffice it to say, cytopathology, particularly cervicovaginal smears, became the object of intense scrutiny and legal proceedings against pathologists and laboratories for alleged failure to interpret the smears correctly, casting a deep shadow on this otherwise very successful laboratory test.

As a consequence of these events, several manufacturers brought about changes in collection and processing of the cervicovaginal smears. The collection methods of cervical material in liquid media, followed by automated processing with resulting "monolayer" preparations, have been approved by the Food and Drug Administration (USA) in 1996. [7, 63]

Diagnostic cytology:

It is based on four basic sampling techniques:

1. Collection of exfoliated cells.

2. Collection of cells removed by brushing or similar abrasive techniques.

3. Aspiration biopsy (FNA) or removal of cells from palpable or deeply seated lesions by means of a needle, with or without a syringe.

4. Intraoperative cytology.

Fine Needle Aspiration Cytology – Techniques and Smear Preparation:

Needles:

Hypodermic, disposable needles with long bevels are well suited for aspiration. The needles may vary in size from 22 to 27 gauge. The choice of gauge depends upon extend to access and quantity to drain according to each case. Larger-bore needles are needed for the evacuation of extremely viscous fluid, so as to collect more material from loosely structured cellular organs (liver) or lesions (melanomas, small cell tumours, and lymphomas). On the other hand, the smaller-bore needles are more effective for sampling tissue with few epithelial cells and extensive fibrosis as they penetrate the stroma more easily and also are more effective in securing the epithelial cell component for example in an invasive lobular carcinoma and fibrocystic change with the dominant fibrous component in the breast.

Syringes and Syringe Holders:

Slip Tip disposable syringes with an eccentric tip are recommended because of the ease of removing and reattaching the needle. A syringe holder can be used when a free hand is needed to stabilize the palpable target during sampling. The syringe holder should hold the syringe firmly, be comfortable to hold, easy to clean, and should not slip easily out of position. It should also allow easy removal of the needle and retraction of the plunger without the need of removing the syringe from the holder. A syringe holder that fits a 10ml rather than a 20-ml syringe is easier to handle as the shorter 10-ml syringe decreases the distance between the hand and the target, making sampling

easier.

A pencil-grip syringe holder developed by Tao and Smith, named The Tao Aspirator is easy to handle and offers a pre-setting of negative pressure to achieve adequate suction.

A supply of clean microscopic slides, coverslips, ingredients, fixatives, alcohol scrubs, for rapid stains, sterile gauze, and band aids must be available before the procedure is initiated. Slides with frosted ends are preferable for proper labelling with pencil for easy identification of the patients.

Patient Selection:

One of the great advantages of FNAC is that it can be applied to almost any patient regardless of physical status, except in deep-seated targets and in patients with clotting disorders. The procedure causes very few, mostly insignificant side effects and only slight discomforts.

Contraindications:

Sampling of carotid body tumours and pheochromocytomas may cause syncope and episodes of acute hypertension. Serious complications in the aspiration of hydatid cysts, include anaphylactic shock. Haemorrhagic diathesis, extremely vascular lesions, and the patient's inability to cooperate may be considered contraindications to deep-seated FNA. Likewise, severe

emphysema, pulmonary hypertension, and conditions associated with severe hypoxemia are also contraindications to FNA sampling of chest lesions.

Patient Preparation:

Procedure should be carefully explained with a discussion of possible side effects. Informed consent must be obtained. For superficial lesions, local anaesthesia is optional. Local anaesthesia is routinely employed when sampling deep-seated targets with the aid of CT or ultrasonic guidance, as these procedures last longer and may be more painful. Moreover several attempts may be needed to position the needle correctly, and the needle tip must often pass through muscle, which is especially sensitive to needle sticks.

A simple disinfection protocol of wiping the skin at and around the biopsy site with an alcohol-soaked swab is sufficient for superficial lesions. For deepseated lesions, it is common practice to cleanse a larger area of skin and use sterile gloves and draping. This facilitates the insertion of the highly flexible needle by allowing the operator to hold onto and stabilize the shaft during insertion.

BASIC ASPIRATION TECHNIQUE:

Palpation of the Target and Planning of the Procedure:

First the lesion should be carefully palpated, and its size and distance from the overlying skin should be assessed. In small lesions (l cm in diameter),

it is desirable to aim for the centre of the lesion. In medium-sized lesions (2 to 4 cm in diameter), it is advantageous to collect samples from two different areas, one to the side of the centre, and another one in the mirrorimage position of the previous aspiration. This approach will yield a more representative harvest. Also, the second sample is extracted from an area not previously disturbed by the needle, thereby decreasing the likelihood of contamination with excessive amounts of blood. In case of very large lesions (>5 cm in diameter), there may be central necrosis, and thus the periphery is more likely to yield diagnostic material.

Immobilization of the Target:

Lesions over 3 cm in diameter can be held in place with the thumb and forefinger. Smaller lesions (1 to 2.5 cm) can be more effectively immobilized between the forefinger and middle finger. Stretching the overlying skin tightly across the lesion further helps immobilize the target. Without moving the fingers, cleanse the skin with an alcohol swab or other disinfectant prior to aspiration.

Insertion of the needle:

Once the target has been secured, the previously assembled aspiration instrument must be picked up and the needle tip inserted into the target.

Suction is applied by retracting the syringe plunger to the 1ml to 2ml mark. Pumping the plunger up and down during the procedure should be avoided, as

it detracts from essential needle movement and does not add to the quantity or quality of the sample.

Aspiration procedure:

Once suction has been applied, the needle tip must be moved back and forth within the target. To collect sufficient material for at least two smears, typically 15 to 20 needle movements are required. Major changes in the direction of the needle must be avoided because they may cause bleeding, unless the procedure outlined below is carefully followed. Using this approach, optimal amounts of tissue will be collected in a short time with minimal bleeding and discomfort. At least one additional aspiration should be performed routinely to ensure representative sampling. However, if the first sample shows unequivocal evidence of a malignant, no further sampling is necessary unless material for special studies is required.

Withdrawal of the needle:

After collecting the sample, release the suction before withdrawing the needle. This allows the collected material to stay within the needle and syringe tip. If suction is maintained while the needle is withdrawn, the collected material will be sucked into the barrel of the syringe and will be difficult to expel. After the needle is withdrawn from the lesion, remove the needle from the syringe and pull back on the plunger. Then reattach the needle and expel the

material onto a glass slide by pushing the plunger swiftly through the syringe. In order to avoid splattering, the tip of the needle should rest on the slide.

ASPIRATION WITHOUT A SYRINGE:

Zajdela et al described a sampling technique that uses a thin needle without suction. The target is identified and immobilized. Then the needle, held by the hub is placed within the target and is moved back and forth to collect small fragments of tissues. The fragments are collected within the shaft of the needle. The hub-opening of the needle should be left uncovered during sampling. The main advantage of this technique is the ease with which the needle can be accurately positioned in the target. The thin needle is also easier to manipulate when it is not attached to a syringe. This simple technique also often enhances the differences in consistency between lesion and the surrounding normal tissue. In addition, sampling without suction may reduce the amount of blood when highly vascular lesions or organs are sampled. Typically, the volume of the harvested material is usually smaller than that obtained by procedures that apply suction. However, the smaller volume may be more representative of the lesion. Sampling without suction may be especially useful in small, highly vascularised targets, such as the thyroid, and in other sites with abundant blood supply. This technique is not recommended for aspirating cystic lesions containing fluid. [80]

SIDE EFFECTS AND COMPLICATIONS:

The most common complication is local hematoma in and around the mass. The bleeding may occur during the sampling or for a few minutes after withdrawal of the needle. The size of the hematoma can be minimized by applying firm pressure to the mass and the surrounding area immediately following the sampling. If the patient has a bleeding disorder or is on type of medications that interferes with blood clotting, the post-aspiration pressure should be extended for up to 10 minutes.

Local infection is extremely rare, and can be managed with antibiotics. No serious infections following FNA of superficial targets have been reported.

Deep-Seated Targets

The complication rates for deep-seated targets are higher than those for superficial targets, and occasional fatalities have been reported. Serious bleeding will occur if the needle tip lacerates the liver or splenic capsule. The risk of laceration is significantly increased if the patient is not able to cooperate with breathing instructions. To minimize the risk of serious haemorrhage, the patient's coagulation parameters should be evaluated before the procedure. It is advisable to temporarily stop medication that interferes with coagulation to reduce the risk of haemorrhage.

BASIC SMEAR PREPARATION TECHNIQUES:

The basic one-step smearing technique is to process a harvest consisting of one or two droplets of semisolid tissue material. The nondominant hand steadies the needle as the needle tip, bevel down, and is touched to the frosted (proximal) end of a clean slide. The harvest (or part of it) is expelled onto the slide in the form of a droplet. The slide is picked up by its frosted end between the thumb and forefinger of the non-dominant hand. The other fingers are used to create a steady platform beneath the slide. A clean slide is then held by its frosted end in the dominant hand. Its lower long edge is placed against the first slide at a 45° to 90° angle proximal to the droplet. This top edge of the slide is then lowered until it touches and then covers the droplet and the two slides are flush. At this point, the material is spread in one smooth motion by pulling the top slide along the entire length of the bottom slide. The movement should be smooth and fairly rapid and not hurried. It is very important to keep the two slides parallel to each other during smear preparation to avoid scraping the bottom slide. As soon as the smear has been prepared, the first (bottom) slide should be fixed. Any delay in fixation will result in air-drying artefacts. The second (top) slide used for smear preparation usually contains no diagnostic material and can be reused to make several smears from the same harvest.

When the droplet is large, the material can be divided to prepare additional smears. For this, two slides are initially positioned as for the onestep smear technique. The top slide is then gently rotated down until it just touches

the droplet, thus picking up a portion of this material. The two slides are then separated. The top slide is kept in the dominant hand and is used to prepare a smear via the one-step method, using a new clean slide as the bottom slide. Finally, a smear is made of the original droplet, again using the one-step technique.

FIXATION AND STAINING OF ASPIRATED MATERIAL:

The most commonly used stains for smears of aspirated material are the Papanicolaou, hematoxylin-eosin, and Romanowsky-type stains.

The Papanicolaou stain requires immediate fixation in alcohol before the smears start to dry. Ethanol (95%) is most commonly used; methanol in a 70% to 95% solution also produces good results.

Smears intended for Romanowsky-type stains are air-dried before the staining procedure is initiated, and can be stored indefinitely in their unstained state.

Rapid Staining Techniques:

A rapid staining technique is valuable for a preliminary evaluation of material before the patient is released. The main purpose is to ascertain that adequate material has been collected. This approach facilitates the decision as to whether to collect additional material for special studies in selected cases. In addition, in many cases a preliminary diagnostic assessment can also be made. Examples: fast version of the Papanicolaou method, the hematoxylineosin stain designed for frozen sections, Diff-Quik or May-GrüenwaldGiemsa stains with the staining time reduced to 2 minutes for each, and toluidine blue, which is so far the fastest.

Advantage of Fine needle aspiration cytology:

(1) Rapidity of diagnosis.

(2) High acceptance.

(3) Cost-effectiveness.

(4) High sensitivity and specificity. [74]

(5) Ability to sample multiple areas at a single go.

(6) Preoperative planning.

(7) Sampling of metastatic as well as the primary site.

(8) Performance of ancillary techniques.

(9) A rapid psychological relief to the patient following a negative diagnosis. [10]

Limitations of Fine needle aspiration cytology:

(1) Results and accuracy are highly dependent on the quality of samples and smears.

(2) Samples obtained with a fine needle may not be representative in case of a heterogeneous lesion or under USG guidance.

(3) Lesions recognised mainly on the specific micro architectural pattern, which may not be sufficiently represented in cytological preparations.

(4) Ancillary techniques like immune markers cannot be done.

(5) Precise cytological criteria have not yet been defined in some rare conditions.

Continuous frequent exposure to a particular category of tumours has been clearly shown to be a major factor deciding diagnostic accuracy. On the

other hand, because such cases will be seen initially in general medical practices and surgical clinics, all cytopathologists must be able to at least categorise the condition and suggest the appropriate referral. This requires a wide knowledge of the range of possible conditions in any given site.

Thyroid Gland:

Sampling of a mass in the thyroid gland is easiest if the patient lies flat on the back, either without a pillow or with a thin pillow under the head. However, if stretching the neck brings the mass forward and makes it easier to palpate, then this is the best position for the patient to be in.

To make sure that the mass is in the thyroid, feel the mass move up and down on deglutition before sampling. Immobilizing a target in the thyroid gland is best achieved by pushing the lesion against the trachea, using the volar aspects of the forefinger and middle finger. The sternocleidomastoid muscle should be pushed to a lateral position and should not be allowed to cover the target. Also, small fragments of muscle may plug the needle, jeopardizing subsequent sampling of the target. Ideally, the angle of the needle should be tangential to the trachea. This way, the needle will be less likely to penetrate the trachea, with

resulting loss of the sample in the barrel of the syringe. The tangential approach also allows easier sampling of small and/or flat lesions close to the trachea. It is important for the patient to refrain from swallowing during the sampling.

By early 2000‟s routine use of FNA for the preoperative diagnosis in thyroid nodules started being the norm. It also helped to reduce the rate of unnecessary thyroid surgery for patients with benign nodules and hence appropriately triage patients with thyroid cancer to appropriate surgery. Before the routine use of thyroid FNA, the percentage of surgically resected thyroid nodules that were malignant was 14%. [(23)] With current thyroid FNA practice, the percentage of resected nodules that are malignant, surpasses 50%.[(24)]

Historically, terminology for thyroid FNA had varied significantly from one laboratory to another, thus creating confusion in cases and also hindering the sharing of clinically meaningful data among multiple institutions. With routine use of FNA in thyroid cases, it became critical that cytopathologists communicate thyroid FNA interpretations to referring physicians in terms that are succinct, unambiguous, and clinically helpful.

To address the terminology and other issues related to thyroid FNA, the National Cancer Institute (NCI) hosted “NCI Thyroid Fine Needle Aspiration State of the Science Conference.” The meeting was organized Dr. Andrea Abati, MD, and took place on October 22 and 23, 2007, in Bethesda, MD. Edmund S. Cibas, MD, and Susan J. Mandel, MD, MPH, served as

moderators. Zubair W. Baloch, MD, PhD, served as chair of the Terminology and Morphologic Criteria committee.

The inspiration for thyroid proposal was the "Bethesda System for reporting cervical cytology" interpretations, first developed at an NCI workshop in 1988 and widely adopted for reporting cervical pap smears. It was expected that the many benefits, clinical and investigational, of the Bethesda cervical terminology would also apply to the Bethesda thyroid terminology.

A uniform reporting system for thyroid FNA would facilitate effective communication among cytopathologists, endocrinologists, surgeons, radiologists, and other health care providers; thus facilitating cytologichistologic correlation for thyroid diseases. It would also facilitate research into epidemiology, molecular biology, pathology, and diagnosis of thyroid diseases, particularly in neoplasia and allow for the easy and reliable sharing of data from different laboratories for national and international collaborative studies.

The Bethesda System for Reporting Thyroid Cytology (TBSRTC):

Category I: Non diagnostic or Unsatisfactory:

All thyroid FNA must be evaluated for adequacy. Inadequate samples are reported as "nondiagnostic" (ND) or "unsatisfactory" (UNS). This applies

to specimens that were unsatisfactory owing to obscuring blood, overly thick smears, air drying of alcohol-fixed smears, or due to inadequate number of follicular cells.

Exceptions included are:

- Any specimen that contained abundant colloid was considered adequate (and benign), even if 6 groups of follicular cells were not identified.

- Whenever a specific diagnosis (eg, lymphocytic thyroiditis) can be rendered and whenever there is any atypia, the specimen is, by definition, adequate for evaluation.

ND/ UNS results occurred in 2% to 20% of cases but ideally should be limited to no more than 10% of thyroid FNAs, excluding samples composed exclusively of macrophages. [27,28]

At the 2007 NCI Conference, it was decided that cyst-fluid-only (CFO) cases should be considered a clearly identified subset of ND/UNS. The significance and clinical value of a CFO result depend in large part on sonographic correlation.

In a study that segregated CFO cases and analyzed them separately, the risk of malignancy for a CFO sample was 4%.[28] The risk of malignancy for

ND/UNS (not including CFO) is 1% to 4%.[27,28,29] A repeated aspiration with ultrasound guidance is recommended for ND/UNS and clinically or sonographically worrisome CFO cases and is diagnostic in 50% to 88% of cases, but some nodules remain persistently ND/ UNS. Excision is considered for persistently ND/UNS nodules because about 10% prove to be malignant.[30] Unless specified as ND/UNS, the FNA specimen is considered adequate for evaluation.

Category II: Benign

- Benign Follicular Nodule (BFN) : refers to an adequately cellular specimen composed of varying proportions of colloid and benign follicular cells arranged as macrofollicles and macrofollicle fragments. The false-negative rate of a benign interpretation is low (0%-3%),[24,31] but patients are nevertheless followed up with repeated assessment by palpation or ultrasound at 6- to 18-month intervals.[32] If the nodule showed significant growth or "suspicious" sonographic changes, a

  repeated FNA can be considered.

- "Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context" and "Consistent with granulomatous (sub-acute) thyroiditis." This is a partial list and does not include a variety of other

benign conditions like infections and amyloid goitre that are occasionally sampled by FNA. Additional benign findings (eg: black thyroid, reactive changes, radiation changes, cyst lining cells) can be mentioned as descriptive diagnoses at the discretion of the

cytopathologist.

- Category III: Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance

  Some thyroid FNAs are not easily classified into the benign, suspicious, or malignant categories. The heterogeneity of this category precludes outlining all scenarios for which an AUS interpretation is appropriate.

- A prominent population of microfollicles in an aspirate that does not otherwise fulfill the criteria for "follicular neoplasm/suspicious for follicular neoplasm." This situation may arise when a predominance of microfollicles is seen in a sparsely cellular aspirate with scant colloid. Alternatively, a more prominent than usual population of microfollicles may occur in a moderately or markedly cellular sample, but the overall proportion of microfollicles is not sufficient for a diagnosis of follicular neoplasm/suspicious for follicular neoplasm.

- There is a predominance of Hürthle cells in a sparsely cellular aspirate with scant colloid.

- The interpretation of follicular cell atypia is hindered by sample preparation artefact, eg: 1) Air-drying artefact with slight nuclear and cytoplasmic enlargement, pale and slightly smudgy chromatin, and/or mildly irregular nuclear contours 2) Clotting artefact with crowding.

A moderately or markedly cellular sample is composed of a virtually exclusive population of Hürthle cells, yet the clinical setting suggests a benign Hürthle cell nodule, eg: 1. Lymphocytic (Hashimoto) thyroiditis 2. Multi nodular goitre

There are focal features suggestive of papillary carcinoma, including nuclear grooves, enlarged nuclei with pale chromatin, and alterations in nuclear contour and shape in an otherwise predominantly benign-appearing sample (especially in patients with Hashimoto thyroiditis or with abundant colloid and other benign-appearing follicular cells).

There are cyst-lining cells that may appear atypical owing to the presence of nuclear grooves, prominent nucleoli, elongated nuclei and cytoplasm, and/or intranuclear cytoplasmic inclusions in an otherwise predominantly benign appearing sample.[33]

A minor population of follicular cells show nuclear enlargement, often accompanied by prominent nucleoli.

Eg: 1. Specimens from patients with a history of treatment with

radioactive iodine, carbimazole, or other pharmaceutical agents.

2. Repair due to involutional changes such as cystic degeneration and/or hemorrhage.

There is an atypical lymphoid infiltrate (in which a repeated aspirate for flow cytometry is desirable), but the degree of atypia is insufficient for the general category "suspicious for malignancy."

Not otherwise categorized: only nodules with atypia of undetermined significance should be placed in the AUS category. Recognizably benign cellular changes (eg, typical cyst lining cells, focal Hürthle cell change, changes ascribed to radioiodine therapy, black thyroid) should not be interpreted as AUS. A moderately or even highly cellular specimen by itself (without significant nuclear or architectural atypia) does not qualify a nodule for an AUS interpretation. An AUS result is obtained in 3% to 6% of thyroid FNAs. [25,30] Higher rates likely represent overuse of this category when other interpretations are more appropriate. The recommended management is clinical correlation and a repeated FNA at an appropriate interval. [25,33] In most cases, repeated FNA can result in a more definitive interpretations, of which only about 20% of nodules are repeatedly AUS.[25] The risk of malignancy for an AUS nodule is difficult to ascertain because only a minority of cases in this category have surgical follow-up. Those that are resected represent a selected population of patients with repeated AUS results or patients with worrisome clinical or sonographic findings. In this selected population, 20% to 25% of patients with

AUS prove to have cancer after surgery, but this is undoubtedly an overestimate of the risk for all AUS interpretations. [25, 30] The risk of malignancy is certainly lower and probably closer to 5% to 15%.

Category IV : Follicular Neoplasm or Suspicious for a Follicular Neoplasm

This diagnostic category helps to identify a nodule that might be a follicular carcinoma (FC) and triage it for surgical lobectomy. FCs have cytomorphologic features that distinguish them from benign follicular nodules. Although these cytomorphologic features do not permit distinction from a follicular adenoma (FA), they are reportable as “follicular neoplasm” (FN) or “suspicious for a follicular neoplasm” (SFN), leading to a definitive diagnostic procedure, usually lobectomy. About 15% to 30% of cases called

FN/SFN prove to be malignant. The majority of FN/SFN cases turn out to be FAs or adenomatoid nodules of multinodular goiter, both of which are more common than FC. Of those that prove to be malignant, many are FCs, but a significant proportion are follicular variants of papillary carcinoma. Cytologic preparations typically have high cellularity, with scant or absent colloid. The hallmark of this diagnostic category is a disturbed cytoarchitecture: follicular cells which are arranged predominantly in microfollicular or trabecular pattern. Cases that demonstrate the nuclear features of papillary carcinoma are excluded from this category. Cellular crowding and overlapping are conspicuous, and the follicular cells are usually larger than normal. Nuclear atypia or pleomorphism

and mitoses are uncommon. A minor population of macrofollicles (intact spheres and fragments) can be present. Conspicuous cellularity alone does not qualify the nodule for a suspicious interpretation. If the sample is cellular but mostly macrofollicular, a benign interpretation is appropriate. Benign follicular nodules often have a small population of microfollicles and crowded groups. If these constitute the minority of the follicular cells, they have little significance and the FNA can be interpreted as benign. A suspicious interpretation is rendered only when the majority of the follicular cells are arranged in abnormal architectural groupings (microfollicles, crowded trabeculae). The general category FN/SFN is a selfsufficient interpretation; narrative comments that follow are optional. In the World Health Organization classification, Hürthle cell adenoma and Hürthle cell carcinoma are considered oncocytic variants of FA and FC, respectively. Studies suggest, however, that follicular and Hürthle cell tumors have different underlying genetics. For this reason, and because they have such distinctive morphologic features, it is helpful to specify that a sample raises the possibility of a Hürthle cell rather than a follicular neoplasm. This interpretation applies to cellular samples that are composed exclusively (or almost exclusively) of Hürthle cells. Oncocytic cells with nuclear features of papillary carcinoma are excluded from this interpretation. A significant proportion of these cases (16%-25%) prove not to be neoplasms but rather hyperplastic proliferations of Hürthle cells in nodular goiter or lymphocytic thyroiditis. About 15% to 45% of nodules are malignant, and the remainder of the neoplasms prove to be Hürthle cell adenomas.

Suspicious for Malignancy:

Most cases of papillary thyroid carcinoma (PTC), can be diagnosed with certainty by FNA. However the nuclear and architectural changes of some PTCs are subtle and focal. This is particularly true of the follicular variant of PTC, which can be difficult to distinguish from a benign follicular nodule. Other PTCs may be incompletely sampled and yield only a small number of abnormal cells. If only 1 or 2 characteristic features of PTC are present, if they are only focal and not widespread throughout the follicular cell population, or if the sample is sparsely cellular, a malignant diagnosis cannot be made with certainty. Such cases occur with some regularity, and they are best classified as "suspicious for malignancy," qualified as "suspicious for papillary carcinoma." Nodules called suspicious for papillary carcinoma are resected by lobectomy or thyroidectomy. Most (60%-75%) prove to be papillary carcinomas, and the rest are usually FAs.

The same general principle applies to other thyroid malignancies like medullary carcinoma and lymphoma, but these are encountered less frequently than PTC. Ancillary testing (eg, immunohistochemical analysis, flow cytometry) in borderline cases is usually more helpful with medullary carcinoma and lymphoma than with PTC.

Malignant:

The general category malignant is used whenever the cytomorphologic features are conclusive for malignancy. Descriptive comments that follow are used to sub classify the malignancy and summarize the results of special studies, if any. Approximately 3% to 7% of thyroid FNAs have conclusive features of malignancy, and most are papillary carcinomas. Malignant nodules are usually removed by thyroidectomy, with some exceptions (eg: metastatic tumours, non-Hodgkin lymphomas, and undifferentiated carcinomas). The positive predictive value of a malignant FNA interpretation is 97% to 99%.

Rabia Basharat et al conducted a comparative study at the Department of Pathology at King Edward Medical University titled „Comparison of FNAC and thyroid scan in solitary thyroid nodule". This study comprised of 50 patients diagnosed clinically with solitary thyroid nodules (STN) and who underwent thyroid function tests and thyroid scan (TS). These patients later underwent FNAC in the department of Pathology, Mayo Hospital. The cases were operated and evaluated for histopathological changes. Thyroid scan revealed that 40 patients (80%) having cold nodules were labeled as suspicious, 10 patients (20%) had hot nodule. On FNAC 23 patients (46%) had benign lesions, 22 patients (44%) had indeterminate lesions and 5 patients

(10%) had malignant lesions. On histopathologic examination, 45 patients (90%) were confirmed to have benign lesions and 5 patients (10%), malignant lesions. After comparison of results of thyroid scan and FNAC with

histopathology, the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of thyroid scan were 80%, 20%, 10%, 90% and 26%, respectively whereas those of FNAC were 80%, 97.7%, 80%, 97.7% and 96%, respectively. The authors concluded that Fine needle

aspiration was a significantly better predictor of malignancy than thyroid scan and resulted in a smaller proportion of excisions for benign nodules.[20]

Sengupta A et al conducted a prospective study to compare the advantage of pre-operative FNAC of thyroid swellings with post operative histopathology to reach a consensus protocol for optimal management of thyroid swellings. The authors studied 178 incidental cases and reported the preponderance of colloid goitre (75.84%) followed by granulomatous thyroiditis. Follicular carcinoma was noted in 7.30% and anaplastic carcinoma in 3.37% cases. Histopathological examination showed predominantly colloid goitre (76.97%), followed by follicular carcinoma (8.99%). The overall prevalence of malignancy was 11.24% diagnosed by HPE, and 9.55% by FNAC. In the FNAC series sensitivity was 90% while specificity was 100%; accuracy was 98.88%. Predictive value of a positive test and negative tests was 100% and 98.75% respectively. The authors concluded that FNAC should be treated as a first-line diagnostic test for thyroid swellings to guide the management though this is not a substitute for HPE as a need to improve

primary healthcare in India. [21]

Sukant Garg et al conducted a retrospective study to evaluate the results of FNAC in the diagnosis and management of thyroid lesions. The authors reviewed FNAC‟s performed on 434 patients over a period of three

years. The cytological results were correlated with clinical features, biochemical investigations, and subsequent histopathological examination and management of the patients. The most frequently encountered lesion was the colloid goitre in 250 (57.60%) cases followed by thyroiditis in 119 (27.41%) cases, ten (2.30%) adenomatous goitres and two (0.004%) thyroglossal cysts.

14 (1.38%) cases were reported as follicular/Hurthle cell neoplasms and 17 (3.91%) as malignant tumours. When compared with the clinical diagnosis, FNAC proved to be an improvement on the diagnosis of thyroiditis and malignancy when compared with that of goitre. FNA revealed a sensitivity of 97%, a specificity of 100%, a positive predictive value of 96% and a negative predictive value of 100%. They concluded that FNAC is a minimally invasive, highly accurate and cost-effective procedure for the assessment of thyroid lesions. It also helped in differentiating lesions that require surgery from those

that can be managed otherwise.[22]

Breast:

Breast cancer is the second most common cancer among Indian females. The cumulative incidence in females until 64 years of age is 1-2%. Following public education programs including self-examination and triple test, the incidence of early detection of breast lump has increased.

Sir James Paget is credited for aspirating malignant cells from a breast cancer patient in 1853. Much of the early experience of aspiration biopsy was not with “fine” needles but with larger bore cutting needles. The popularity of this simple procedure has largely been because of its cost effectiveness as well as the inherent qualities of the procedure itself such as low complication rate, rapidity, and high diagnostic accuracy. Palpable lesions can be effectively biopsied using a thin needle (23 gauge or smaller) without radiologic guidance. However, with the current trend of detecting smaller, non-palpable lesions, radiologic guidance (mostly ultrasound) is needed to adequately sample smaller lesions. The “triple diagnostic approach,” which consists of palpation, radiologic findings, and cytopathologic analysis on fine needle aspiration (FNA), is applicable to benign, pre neoplastic, borderline, and malignant diseases of the breast.

Ahmed HG et conducted a descriptive longitudinal study at the University of Khartoum, Khartoum, Sudan in which they did FNA for 200 patients with palpable breast lesions and compared findings with tissue biopsies taken later. Data were analyzed using a computer's SPSS program.

Pearson chi-square test was used for statistical analyses. The diagnoses of the 200 breast FNAs were as follows: 61 (30.5%) were malignant, 5 (2.5%) were suspicious, and 134 (67%) were benign lesions. Subsequent histopathological examination was performed on 61 (100%) patients with malignant lesions, 5 (100%) of suspicious, and 65 (48.5%) patients of 134

patients with benign lesions. FNAC revealed a 92.6% sensitivity, a 95.2% specificity, a 95.5% positive predictive value, and a 92.2% negative predictive value. They concluded that FNAC of breast lesions was sensitive, specific, and highly accurate as the initial investigation of palpable breast lesions in a population of low resources and without screening program to diagnose breast cancer. [11]

Pandey A. et al conducted a prospective study at the department of Pathology, L.N. medical college, Bhopal, India from January 2016 to December 2016.

FNAC of 300 cases of palpable breast lesions was done and reported by expert pathologist. The histopathological specimen when available was reported by another pathologist without prior knowledge. Sensitivity, specificity and accuracy of FNA diagnosis was then analyzed. A total of 300 cases of breast lesion were diagnosed on FNA, out of them histopathological correlation was available for 150 cases. Benign breast lesions were more common in younger patients in 11-30 age group and malignant breast lesion were more common in the 41-60 years age group. Benign breast lesions were found in 215 cases (71.66%); among which fibroadenoma (41%) was the commonest lesion observed. Malignancy was observed in 63 cases (21%); among them, Ductal carcinoma was the predominant lesion (17.66%). The sensitivity, specificity and diagnostic accuracy of FNAC for malignant lesion was found to be 98.3%, 98.9% and 98.7% respectively. They concluded that FNAC is an effective and valid tool as first line diagnostic modality in preoperative diagnosis of malignant and benign breast lesions

Ashish Kosthi et al conducted a retrospective hospital based study at the department of Pathology, Gandhi Medical College, Bhopal over a period of two and a half years. It included 531 patients with breast lump. FNAC was done, 31 (5.84%) cases were not satisfactory and remaining 500 (94.16%) were satisfactory for cytological diagnosis. Out of 500 cases, benign lesions were 358 (71.60%), malignant lesions were 87 (17.40%), inflammatory

lesions were 41 (8.20%) and suspicious category include 14 (2.80%). Fibroadenoma was the most common benign lesion and ductal carcinoma was the common malignant lesion. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of FNAC was 98.13%, 100%, 100%, 98.98% and 99.34% respectively. They concluded that FNAC of the breast lump is a simple, safe, economical, and rapid diagnostic procedure which can be used routinely on OPD basis, because the cytopathological examination of these lesions before operation or treatment serves as an

important diagnostic modality.[12]

Katherine T. Morris et al conducted a diagnostic test study at the university hospital multidisciplinary breast clinic. They studied 479 women with palpable breast lesions and evaluated them by triple test score (TTS). All lesions with TTS less than or equal to 4 were benign on clinical follow up. Lesions with TTS greater than or equal to 6 (n=130) were confirmed to be malignant on biopsy. Confirmatory biopsy was required only for the 8 % of masses that received a TTS of 5. They concluded that TTS reliably guides evaluation and treatment of palpable breast masses. [15]

Problems with FNAC:

The performance and interpretation of breast FNA require adequate training and experience. Correlation with subsequent biopsies and clinical

follow-up is mandatory in order to improve the diagnostic yield and accuracy of the procedure. Over the years FNA as a means to investigate palpable breast lesions became popular, so did the concern over false positive and false negative diagnoses among cytopathologists as well in the setting of malpractice lawsuits.

Scott Boerner et al conducted a retrospective study at The University of Texas M.D. Anderson Cancer Center to better understand the significance of epithelial cellularity, false-negative FNA samples from palpable breast lesions. They reviewed 4455 aspirates of palpable breast masses for specimen adequacy in the form of epithelial cell clusters. Aspirates were classified as being adequate if a total of six or more epithelial cell clusters(ECC) each comprising ofat least five to ten well preserved cells were present on all slides or as inadequate if fewer than six ECC‟s were present. From the 4455 aspirates of palpable breast masses, 51 false-negative aspirates were identified, 41 of which were available for review. No interpretative errors were identified. Twenty-one FNAs (51.2%) were classified as adequate and 20 FNAs (48.8%) as inadequate. The adequate false-negative aspirates contained between 8 to 100 ECCs. A comparison of adequate and inadequate false-negative specimens showed no significant differences in the mean age of patients (56.4 years vs. 57.8 years), the mean number of FNA passes (3.7 passes vs. 3.0 passes), the mean palpation size of the lesions (2.8 cm vs. 2.9 cm), or the mean pathologic size of the lesions (2.1 cm vs. 2.2 cm). Cases of invasive lobular carcinoma were more

common in the false-negative smears with fewer than six ECCs. They concluded that including the number of ECCs as a parameter of adequacy could reduce the rate of false-negative FNA diagnoses of palpable breast masses by approximately 50%. However, the presence or even abundance of ECCs does not eliminate the potential for a false-negative cytologic diagnosis. Cytologic diagnoses must be correlated with clinical and imaging findings (the triple test) to reduce the rate of falsenegative cases, but benign triple test results do not entirely exclude the possibility of carcinoma, and such cases require periodic follow-up. [18]

Lee KR et at conducted a 3 year comparative study at the Medical Centre Hospital of Vermont where they analysed all the 503 FNA of breast that was performed. There were 93 aspirates diagnosed as "positive," all of which were from patients eventually shown to have cancer. However, there were 38 patients with primary carcinoma in which the FNA was not diagnosed as positive, for a diagnostic failure rate of 31.4%. In order to determine the possible effect of technique as practiced by an experienced aspirator in diminishing such diagnostic failures, they compared 190 aspirates obtained by a single individual with 193 aspirates obtained by 15 individuals in the same community. For the single experienced aspirator, the technical failure rate was 9.8% whereas in the group with many aspirators it was 45.9%. They

concluded that although fine needle aspiration of the breast is considered easy to perform, skill on the part of the aspirator is important for satisfactory results.(14)

Scopa, C. D et at conducted study at the Departments of Pathology and Surgery, University of Patras Medical School, Patras, Greece. They studied 39 cases in which FNA posed diagnostic problems. They concluded that these problems could be attributed to sampling errors (71.8%), to the criteria of adequacy we use at our institution (2.5.6%), and to interpretation (2.6%). The nature of the breast lesion (68%) was the most common cause of inadequate sampling, followed by the experience of the aspirator (32%).(13)

Layfield, L. J. et al studied a subgroup of 183 cases with known outcome, drawn from a series of 1779 cases, to determine the minimum number of cell clusters necessary to ensure that adequate cellular material was present for accurate diagnosis. The series included 21 cases cytologically diagnosed as false-negative, 75 cases that had been correctly identified as benign, 47 cases cytologically designated as atypical, and 40 cases that on initial review had been correctly identified as malignant. In a semi blind fashion, the smears from each case were assigned to low, medium, and high cellularity categories. Low cellularity was defined as 10 or fewer cell clusters, moderate cellularity was defined as 11-30 clusters,

and high cellularity was defined as more than 30 clusters. A cell cluster was defined as five or more cells. Within the low cellularity group, exact numbers of cell clusters and the presence of individual cells were recorded. The presence of bipolar cells was used as an adjunct criterion for specimen adequacy, and the bipolar cells in each of 10 × 200 fields were counted. Cellularity was then correlated with diagnostic accuracy. Using a cutpoint of a cumulative score of 6 or more cell clusters or the prominence of bipolar cells (≥10 in each of 10 medium-power, ×200 fields) for assessment of specimen adequacy, a false-negative rate of 1.5%, associated with an unsatisfactory rate of 20.2%, was obtained. The authors concluded that hat the sampling false-negative and unsatisfactory rates can be minimized by selecting a cut point for satisfactory smears at a level of 6 or more cell clusters (cumulative total) or the presence ≥ 10 intact bipolar cells per 10 medium-power fields (×200). Use of these criteria will decrease the false-negative rate of sampling in epithelial lesions of the breast. A falsenegative rate of approximately 1.5% was obtained in association with an unsatisfactory rate of 20.2%. Using a cut point of 1 or more cell clusters, a false-negative rate of 2.1%, associated with an unsatisfactory rate of 13.7%, was obtained.[19]

Overall, breast FNA is enormously successful, with an overall diagnostic sensitivity ranging from 80% to 100%, with specificity over 99%. In the modern era, breast FNA has been confronted with new roles and challenges. It is now routinely expected that breast FNA will provide an

accurate diagnosis, analyse the biologic behaviour of the tumour, supply biomarker information such as oestrogen/progesterone receptor status, comment on cell proliferation index, and determine prognostic indicators such as Her2neu expression. These expectations can only be met if an adequate sample is obtained and the pathologist is on site to triage the material for processing.

Several studies have described the use of stereotactic mammography devices for sampling non palpable breast lesions. [72,73]

High-resolution ultrasound is becoming more common as a diagnostic technique in breast and other organs. It is also a very valuable tool that can be utilized to guide the needle tip into the target. It allows visualization of the needle tip, in real time, as it moves through the target and secures the sample.

This approach provides additional evidence that the sample is truly from the correct target area. Ultrasound guidance is particularly suited for non-palpable breast masses that are visible on ultrasound.

Although most cytopathologists and clinicians who use fine-needle aspiration (FNA) biopsy in their practices are aware of the relationship between expertise in microscopic interpretation and diagnostic accuracy, the importance of sample quality and smear preparation is less well recognized. Several studies have shown that training and experience in obtaining and

preparing the samples play a major role in the efficacy of the method. [15,75,76] It is much easier to interpret FNA samples microscopically if they are abundant, representative, and well prepared. Optimal samples will contribute greatly to a very high degree of diagnostic accuracy. Thus, operators who are well trained in the sampling technique of various body sites will serve patients

better. [77]

Sampling issues encountered FNAC:

1. Cells not collected on sampling devices.

2. Cells collected but not transferred to slide.

Interpretive issues:

1. Abnormal cells present on slide but either not seen or misinterpreted due to blood / mucus / air drying artefact.[9]

Liquid based cytology:

Literally means „the study of cells through a liquid medium".

For many years, efforts have been made to develop methods that would enhance the sensitivity and specificity of the Papanicolaou smear. Emphasis has been placed on creating automated screening machines whose success depends on a representative sampling of cells on standardized slides containing a monolayer of well-stained, well-preserved cells.

From this research and development, liquid-based gynecologic specimen collection has evolved. Its proponents argue that liquid-based preparations outperform conventional smears because of improved fixation, decreased obscuring factors, and standardization of cell transfer. In direct smears, the cells are not transferred in a representative fashion and that up to 90% of the material scraped from the cervix may be discarded with the sampling device. With liquid-based collection, the sampling will be representative and operator-dependent variation will not occur since processing is controlled by the laboratory.

SurePath (TriPath Imaging, Inc, Burlington, NC) and ThinPrep 2000 System (Cytyc Corp, Marlborough, MA) are two such systems approved by the FDA for cervicovaginal testing in 1996. With both methods, the sample is collected in the conventional manner with one of the brush instruments but, instead of being spread onto a glass slide, it is transferred to a vial of fixative.

In the SurePath method, the sample is vortexed, strained, layered onto a density gradient, and centrifuged. Instruments required are a

computercontrolled robotic pipette and a centrifuge. The cells form a circle 12.5 mm in diameter.

The ThinPrep method requires an instrument and special polycarbonate filters. After the instrument immerses the filter into the vial, the filter is rotated to homogenize the sample. Cells are collected on the surface of the filter when a vacuum is applied. The filter is then pressed against a slide to transfer the cells into a 20 mm diameter circle.

Both methods result in a well preserved approximate monolayer of cells, with a background devoid of blood and mucus.

The use of automatic monolayer devices for the preparation of nongynecologic material is becoming popular. AutoCyte Prep, now called SurePath (TriPath, Inc), and the ThinPrep Processor (Cytyc Corp) have both been approved for preparation of nongynecologic material by FDA.

ADVANTAGES OF LBP:

1. Almost 100% of the collected cells are captured, processed, and reviewed.

2. Immediate liquid fixation prevents artifacts, such as air-drying.

3. Easier to review slides, Smaller screening area (TP, 20-mm and SP, 13mm).

4. Preparatory technique reduces debris, cell clumps and obscuring elements, cleaner background.

5. Significantly fewer unsatisfactory cases.

6. Homogenized specimen - Increased detection of high-grade squamous intra-epithelial lesions and above.

7. Ancillary testing such as reflex human papillomavirus (HPV) test and other molecular tests (Chlamydia/gonorrhea), immunocytochemistry can be performed from the residual material.

8. Potential for processing residual material as a cell block.

Michael et al (2001) noted that SurePath offers a somewhat better preservation of architecture of cell clusters and cellular integrity than ThinPrep.

Kurtyn and Hoerl cautioned against the indiscriminate use of thin layer technology. Serious reservations about the use of this technique were also expressed by Michael and Hunter (2000) who also pointed out that the ThinPrep technique leads to artifacts and diagnostic pitfalls. [7]

Salhadar et al concluded that routine use of the ThinPrep technique for aspiration biopsy is diagnostically not justified. [78]

Papillo et al conducted a study whereby cell yields on cytologic preparations made in the Cytospin II cytocentrifuge and the ThinPrep Processor was compared. Slides were prepared by each method using calibrated volumes (25 microliters) of cell suspensions from 13 non gynecologic specimens. Cell counts for each slide were calculated by counting cells in predetermined fields using a gridded reticle at 40 x magnification, then extrapolated to the total surface area of the preparation. The cell counts demonstrated that when processing equal amounts of cell suspension, the ThinPrep method retained three times as many cells as the cytocentrifuge method. The ThinPrep method, with a higher rate of cell recovery, may provide a valuable tool toward more accurate cytological diagnosis, particularly for cytology samples with small numbers of cells.[35]

Martin H. Luu et at conducted a study in which they compared preoperative definitive diagnosis of PTC in FNA prepared using Thinprep(TP) and conventional smears and FNA prepared with Thin Prep alone. Cytological diagnosis of positive for malignancy was correct in 98.8% of TP + CP cases and in 100% of TP cases. Papillary thyroid carcinoma cases were definitively diagnosed in 53.1% of T-FNAs prepared by TP + CP compared with 34.4% of T-FNAs prepared with TP alone. They concluded that ThinPrep as a sole preparatory technique does not improve the usefulness of thyroid FNA as a screening test. However, combining Conventional preparation and Thinprep increases the rate of definitive cytologic diagnosis of malignancy in papillary

thyroid carcinoma.[75]

Pranab Dey et al conducted a study to compare the various cytological features on ThinPrep 2000 and conventional preparation specimens from FNAC material by a semiquantitative system. They studied 71 consecutive cases, first pass was used for conventional smear preparation, second mass for TP preparation. TP preparations contained adequate diagnostic cells in all cases and were tangibly superior to CP preparations. The authors concluded that it was easier and less time consuming to screen and interpret TP preparations because cells are limited to smaller areas on clean background,

with excellent nuclear and cytoplasmic morphology.[36]

Over all technical quality was reported to be improved by ThinPrep processing when compared with direct smears on split FNA specimens due to cleaner background and better monolayer formation.[36,37]

Disadvantages of Automated LBP:

1. Initial high cost for setup.

2. Retraining of technician and cytopathologist.

3. Some cytologic details are different in LBC compared with direct smears.

Some authors, however, advised caution to avoid diagnostic errors when interpreting ThinPrep slides due to the increased incidence of the following cytologic artifacts : disruption of tissue fragments, formation of cell clusters, aggregation of lymphocytes, cellular shrinkage, attenuation of nuclear details, and exaggeration of nucleolar prominence.[37,38] In comparison with SurePath processed specimens, ThinPrep slides demonstrated increased cellular shrinkage, flattening, and fragmentation of large cellular sheets and

nuclear chromatin patterns were reportedly more difficult to evaluate.[38] SurePath slides were also found to contain larger branched 3-

dimensional tissue fragments.[38]

Claire W. Michael et al conducted a comparative study to assess the diagnostic accuracy and different cytomorphological alterations produced by ThinPrep and TriPath PREP liquid-based preparations in non-gynaecologic specimens. They studied 10 urine samples, 4 serous fluids, and 7 fine-needle aspirates (FNAs) prepared by both techniques. FNAs represented one each of: Hashimoto's thyroiditis (HT), hyperplastic colloid nodule (HCN), Hodgkin's lymphoma, liposarcoma, chondrosarcoma, squamous-cell carcinoma (SCC) metastatic to the lymph node, and carcinoid tumor. All 5 participants, none of whom had prior knowledge of the clinical history or histologic diagnosis, reviewed and interpreted the slides.

Both techniques produced a clean background and were equally accurate in urine sample, serous fluids, and three FNAs. TriPath was slightly more accurate in four FNAs: HCN and HT wherein colloid and lymphocytes were better represented, SCC wherein keratin and malignant cells were more readily identified among lymphocytes, and carcinoid which were easier to evaluate on TriPath due to the less cellular shrinkage and more dispersion of cells between aggregates. ThinPrep preparations had more cell shrinkage, and the chromatin was harder to evaluate. Both techniques produced artificial aggregations of lymphocytes, but TriPath had a more evenly dispersed singlecell population between the aggregates, hence rendering them easier to evaluate for atypia. ThinPrep produced fragmentation of large sheets that were flattened, while TriPath contained larger branching sheets in a threedimensional (3-D) configuration. ThinPrep produced a true monolayer of cells that were all spread at the same plane, while in TriPath the cells were spread at slightly different planes, requiring frequent focusing of the viewed plane.[38]

While both techniques are acceptable for diagnostic purposes, they both introduced new cytomorphologic alterations that pathologists need to recognize. TriPath seems superior to ThinPrep in FNAs specimens where preservation of architecture and cellular integrity are important.[38]

Kalpalata Tripathy et al conducted a prospective study on 110 cases to assess the diagnostic accuracy of liquid–based cytology versus

conventional smears in fine needle aspiration samples. They studied 30 cases of breast, 40 of lymph node, 10 of salivary glands, 18 of thyroid and 12 of bone and soft tissue. In each case, two passes were performed. The first pass was for conventional preparation (CP) and the second pass yielded material for thinprep (TP) preparation. Both CP and TP smears were compared for cellularity, background blood and necrotic cell debris, cell architecture, informative background, presence of a monolayer of cells and nuclear and cytoplasmic details by a semiquantitative scoring system. Diagnostic accuracy was better in LBC smears compared with CP smears due to lack of background debris and better cell morphology, which was performed according to Wilcoxon's signed rank test, yielding a P-value of <0.001. However, in some cases, because of a decrease in cell size, clustering and altered background in LBC, a support of CP was essential. The authors concluded that LBC performed on FNA samples can be a simple and valuable technique. Only in few selected cases, where background factor is an essential diagnostic clue, a combination of both CP and TP is necessary.[8]

CYTOLOGY OF LBC-PROCESSED THYROID LESIONS:

BENIGN LESIONS:

The morphologic picture of LBC differs from CS in two aspects:

(a) The cells on the slide are a mono layered representative sample of the entire material collected in the vial with a variable amount of cells which remains in the preservative solution.

(b) The automated process causes some changes in both cellular and background morphology. One of the most important change, occurring in LBC slides, is the appearance of the fragmented colloid which is present as small droplets in the background of a benign nodule with a quantitative detection whereas in CS the colloid usually does not

require a quantization.[7,70]

The LBC picture of a thyroiditis is similar to CS with the exception of the amount of lymphocytes in the background which can be higher than normal because of the spinning of the material before the automated process. When a thyroiditis is suspected, the detection of lympho-epithelial clusters in an inflammatory background is the pivotal clue for the diagnosis and warrants a simple follow-up for the patient.[46]

Charles V. Biscotti et al analysed 41 samples of ThinPrep and Conventional preparations of thyroid FNAC for diagnostic accuracy,

cellularity pattern of colloid, nuclear and cytoplasmic detail. Of these were 25 colloid nodules, 6 papillary carcinomas, 4 follicular adenomas, 2 minimally invasive (encapsulated) follicular carcinomas, 3 Hashimoto's

thyroiditis, and 1 Grave's disease. Both techniques identified seven of the eight carcinomas with the minimally invasive follicular carcinomas categorized as hyper cellular follicular nodule, possibly malignant (HCFN). One papillary carcinoma wasclassified as a HCFN by both TP and CS techniques. The four follicular adenomas were classified as HCFN based on the TP slides. One oxyphilic follicular adenoma, associated with focal lymphocytic thyroiditis, was misinterpreted as Hashimoto's thyroiditis on a conventional smear. Three colloid nodules were interpreted as HCFN based on the TP slides. Two of these were similarly classified based on the conventional smear. ThinPrep slides contained less colloid and the colloid occurred as droplets rather than a diffuse pattern. TP slides had better nuclear detail but more often disrupted cytoplasm. The authors concluded that the TP process does alter some cellular features; however, similar diagnostic accuracy was experienced with the TP and conventional smear preparations.[40]

FOLLICULAR-PATTERNED LESIONS:

Diagnosis of follicular neoplasms (FN) in LBC versus CS is based upon the identification of micro follicles made up of medium-sized thyrocytes in scant colloid.

(1) Lesions with high cellularity but monomorphic cells and occasional enlarged nuclei.

(2) Lesion which is mostly follicular-structured and made up of mediumsized thyrocytes with rounded nuclei and central nucleolus (Follicular Neoplasm) with a malignancy risk between 20 and 30%. The action, although debated in the literature, results in the surgical removal of the lesion which could histologically correspond to both a follicular adenoma or an adenomatous nodule in a goiter (70–80% of cases) but also follicular carcinoma or a follicular variant of a papillary carcinoma (PC) cannot be ruled out only on morphology. The same diagnostic criteria and therapeutic action is applied to the FN composed mostly by oxyphilic (or Hurthle) cells which is defined when follicles are made up of more than 80% of oxyphilic cells and it should be included in the FN category. The colloid amount may be scant (but sometimes is abundant) and features of old haemorrhage (hemosiderin-laden histiocytes) may coexist

(3) Follicular-structured lesions, composed of thyrocytes with elongated and clear nuclei, sometimes with grooves and peripheral nucleoli without papillae, psammomatous bodies, or nuclear pseudoinclusions with a risk of malignancy ranging between 50 and 70%. This category warrants the surgical removal of the nodule as a follicular variant of a PC is very likely to be found at the histological examination (more than

90% of cases).

MALIGNANT TUMORS:

The cytological diagnosis of thyroid malignancy does not differ substantially in LBC preparations as the clear background facilitates the identification and characterization of the cellular details. The most important malignant tumor which can be appropriately identified on LBC preparations is PC which is easily when nuclear pseudoinclusions are detected. Papillary structures and psammoma bodies are seldom identified. In the earlier reports, the difficulty in detecting the distinctive nuclear features of PC was one of the most important objections against the adoption of the thyroid LBC cytology. Medullary thyroid carcinoma (MTC) is a difficult cytological diagnosis.[41]

The LBC technique offers the opportunity to detect the calcitonin expression in the neoplastic parafollicular cells and their concomitant negativity for thyroglobulin. Anaplastic thyroid carcinoma (ATC) is seldom seen in routine thyroid cytology. TLBC picture of ATC usually shows a background of necrotic debris with small clusters of large round or spindle cells with pleomorphic nuclei and prominent nucleoli which stains positive for cytokeratins (useful to confirm the epithelial origin) and negative for thyroglobulin and TTF-1. The LBC diagnosis of the large cell variant of malignant non-Hodgkin lymphoma usually does not constitute a problem and relies on the immunocytochemical expression of LCA, CD20, bcl-6, and other lymphoid antigens. Lung, breast, kidney, large bowel, and laryngeal metastatic carcinomas to the thyroid gland may occasionally present as a single nodule mimicking a primary tumour in which necrotic debris or

hemorrhagic material and clusters of neoplastic cells with features of adenocarcinoma or squamous cell carcinoma are detected on Cs and ThinPrep2000™.

Liquid-based preparations (LBPs) have been particularly studied in thyroid cytology. The reason for such an interest is related to the critical importance of fine-needle cytology in the discrimination between nodule‟s candidate to surgery from lesions which might be treated clinically. In this setting LBC plays an important role in both achieving a diagnosis and providing material for additional techniques (immunocytochemistry, ICC; molecular analysis, MA; flow cytometry, FC). [37] Several studies have demonstrated the reliability of LBPs applied to thyroid nodules: from the classic studies[37,38,40,42,45] to the more recent papers from Europe and the USA.[43,44]

Artefacts more commonly seen with Non gynaecological LBPs:

Cellularity	Nuclear	Background
Cellular shrinkage	Increased naked nuclei	Loss of adipose tissue, stroma, mucin and colloid.
Disruption of tissue fragments	Decreased nuclear chromatin details	Colloid that appears as dense droplets.
Flattening and fragmentations of large cellular sheets	Attenuation of nuclear grooves and pseudo inclusions	Aggregation of lymphocytes.
Formation of cell clusters	Exaggerated nucleolar prominence	
Artificially increased single epithelial cells		

Manual Liquid Based Cytology:

Arul .P conducted a cross-sectional study, wherein 100 FNA samples from various anatomical sites were evaluated using MLBC and CS preparations. Cellularity, blood, informative background, monolayers, cell architecture, cytoplasmic, and nuclear preservation were compared with MLBC and CS preparations by Wilcoxon signed rank test. MLBC

preparations were identified to be superior to CS preparations in view of absence of blood and debris ($P = 0.001$), presence of monolayers ($P < 0.001$), and preservation of cytoplasmic ($P = 0.001$) and nuclear details ($P = 0.001$). However, no statistically significant differences were found between MLBC and CS preparations with regard to cellularity ($P = 0.157$), informative

background (P = 0.083), and architecture (P = 0.739). The author concluded that MLBC preparations in FNAC are a safe, easy, and less time-consuming procedure, and it may have promising diagnostic value in the evaluation of FNA samples from various anatomical sites. However, the use of both MLBC and CS preparations is recommended to achieve optimal diagnostic yield.[47]

Prathima S. et al conducted a prospective study to assess the diagnostic efficacy of Manual LBC in FNAC samples. A total of 60 FNA samples from various anatomical sites were evaluated. Smears were made from conventional preparation (CP) and manual LBC (MLBC) preparation. Both CP and MLBC preparations were compared for cellularity, background, monolayers, cell architecture, cytoplasmic and nuclear details by a semiquantitative scoring system. The diagnostic accuracy was better in MLBC compared to CP in view of absence of blood and debris, presence of monolayers and preservation of nuclear and cytoplasmic details. However, with regard to cellularity, informative background and cell architecture there was no statistical significance. The authors concluded that MLBC performed on FNA samples can be safe, cost effective promising diagnostic technique in combination with CP to achieve high diagnostic yield. [48]

Pawar .P.S. et al compared 50 cases examined by CS and MLBC prepared from cells trapped in needle hub. Direct smears and MLBC smears were compared for cellularity, background, cellular preservation, and

nuclear preservation. Slides were diagnosed independently by two cytologists with more than 5 years‟ experience. Standard error of proportion was used for statistical analysis. Cellularity was low in MLBC as compared with

conventional smears, which is expected as remnant material in the needle hub was used. Nuclei overlap to a lesser extent and haemorrhage and necrosis was reduced, so cell morphology can be better studied in the MLBC technique. MLBC technique gives results comparable to the conventional technique with better morphology. In a set up where aspirators are learners, this technique

will ensure adequacy due to remnant in needle hub getting processed. [52]

Mahinder K. et al compared CS and MLBC in 100 women with breast carcinoma. Sensitivity and specificity for both MLBC and CS was 95.2% and 100% respectively. Positive predictive value and negative predictive value for MLBC and CS was 100% and 96.9% respectively. Oestrogen receptor analysis performed on five unstained MLBC smears as well on biopsy sections of the same five cases were reported as negative. The authors concluded that Manual Liquid based cytology can be safely used as an important adjunct to

conventional FNA. Liquid based cytology ensures better cellular preservation, less cell overlapping and elimination of blood and excessive

inflammation compared to CS. Manual liquid cytology is a technique which can be as

effective a diagnostic tool as CS in low-resource settings like India. Utilization for ancillary tests such as ICC for hormonal receptors and molecular biology is

an additional advantage of MLBC. [51]

Bandoh, N et al compared CS with LBC on 165 patients with cervical lymphadenopathy.81 (49.1%) were diagnosed as benign lymph node and 84 (50.9%) were malignant diseases including 37 (22.4%) of metastatic carcinoma except for thyroid carcinoma, 30 (18.2%) of metastatic thyroid carcinoma, and 17 (10.3%) of malignant lymphoma. The overall statistical values including sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the CS were 75%, 100%, 100%, 78.9%, and

87.1%, respectively, whereas those values for LBC were 91.2%, 100%, 100%, 90.7%, and 95.3%, respectively. The authors concluded that the sensitivity of LBC for malignant diseases tended to be higher than that of CS cytology [49]

Surabhi et at compared the efficacy of MLBC versus CS in smears taken from oral cavity in 21 patients using cytobrush. MLBC technique produced a significant number of satisfactory smears with regard to cell distribution, clarity/resolution, staining characteristics and background/artefacts

compared to conventional methods. The authors concluded that MLBC is a cost-effective cytological technique that may produce oral smears with excellent

cytomorphology and longer storage life. [50]

METHODOLOGY:

The present study was conducted in the Department of Pathology, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Kanyakumari District, Tamilnadu. In the present study the samples were taken from the Department of General Surgery, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Kanyakumari District, Tamilnadu. The specimens in this consisted of pap stained smears prepared conventionally and using UPREP LBC kit.

a) Study design: Comparative Study.

b) Approximate total duration of the study: One and half years.

c) Number of Samples to be studied: Two groups.

Group one: Specimen for conventional smear.

Group two: Specimen for UPREP liquid based preparation smear.

d) Detailed description of the groups:

All men and women in the age group of 20-60 years attending the General Surgery OPD of SMIMS, Kulasekharam, during the period of January 2016 to June 2017.

e) Sample Size of each group:

55

f) Total sample size of the study:

110

g) Scientific basis of sample size used in the study:

$$n = \frac{Z_1\sqrt{[2P(1-P)]} + Z2\sqrt{P_1(1-P_1) + P_2(1-P_2)}]^2}{(P_1-P_2)2}$$

Where

P_1 = Probability of outcome in cell prep plus =73% (Yong Moon-Lee) P_2 = Probability of outcome in Thin prep= 50%

Level of Confidence = 95% [7]

Level of Power of test=80%

Z_1 = Z value associated with set level of alpha = 1.644

Z_2 = Z value associated with set level of beta = 0.841

Hence Sample size = 54.28 ≈55

h) Sampling Technique: convenient sampling

i) Ethics Committee Approval:

The study was approved by the Institutional Human Ethics Committee with reference number SMIMS/IHEC/2015/A/11

j) Inclusion Criteria:

Patients attending General Surgery OPD of SMIMS with palpable lesions in thyroid, breast and lymph node who were sent for fine needle aspiration study.

k) Exclusion Criteria:

Those that are not willing to participate.

Those patients who have undergone chemotherapy / radiotherapy.

l) Method of collection of data and procedure:

Patients attending the General Surgery OPD at SMIMS who have been advised fine needle aspiration will be selected. After a detailed history, and thorough clinical examination, the skin over the area to be sampled is disinfected using alcohol swab. FNA is done using standard disposable syringes fitted with 23G needle. The lesion is palpated and fixed between the left index and middle finger (in case of small lesions) of the examiner. Needle positioned within the target tissue. Plunger is pulled to apply negative pressure, needle moved back and forth inside target lesion. Negative pressure is released while needle remains in target tissue and then the needle is withdrawn. Needle then detached and air drawn into syringe. One small drop of sample is blown onto a microscopy slide and then the smear is made. Then it is immediately placed in alcohol for fixation. This is the first pass and this slide will be stained with pap stain (conventional preparation). The procedure is repeated, however instead of air, preservative solution is drawn into the syringe. The needle with aspirated material is attached to syringe, plunger is pushed down gently to expel the material along with preservative into a conical plastic screw capped container. The syringe is washed two to three times with the preservative to make sure no material is present in the syringe.

The sample is then kept as such for 30 minutes. The preserved sample is then centrifuged in a Swinging type centrifuge at 1000 RPM for 10 minutes. Discard the supernatant and then agitate the pellet to get a homogenous sample. Add one or two drops of preservative solution over the pellet and mix well again. Add a drop of fixative solution to the slide and take 50µl of the diluted sample and smear over them. Allow the smear to air dry and proceed for staining.

m) Parameters to be studied:

Screening of Smears by Microscopy:

- Cellularity
- Background
- Monolayer
- Cellular morphologic change (architectural and cytoplasmic distortion, cytoplasmic vacuolation, cellular elongation, folded cytoplasmic borders).
- Nuclear changes (nuclear hyperchromasia, coarse chromatin, prominent nucleoli, irregular nuclear borders, atypical mitosis).
- Inflammatory infiltrate

n) Method/Technique/Instrument/Reagent/Kit etc. used along with their manufacturing source details:

- Tissue sampling
- Light Microscope

UPREP LBC kit marketed by Regenix Drugs LTD, #11, First Floor, Loganathan Nagar, 3rd Street, Choolaimedu, Near MMMDA Metro Station, Chennai- 600094. E mail: www.info@uprepindia.com

Chemicals used for staining procedures:

Processing chemicals and materials:

- Xylene.
- Alcohol (different grades)
- DPX
- Frosted microslide 75mm long, 25mm wide, and 1.25mm thick.
- Microscopic coverglass 22mmx22mm.

Procedure for Papanicolaou Staining:

1. 70 % Isopropyl Alcohol – 1min
2. 50 % Isopropyl Alcohol – 1min
3. Distilled water – 1 min
4. Harris Hematoxylin – 1min and 30 seconds
5. Tap Water – 1 min
6. 0.5% HCL in 70 % Isopropyl Alcohol – 1 dip
7. Distilled water – 5 min
8. Ammoniated Alcohol Solution – 1 min.
9. Running tap water – 15 min
10. 50 % Isopropyl Alcohol – 1min
11. 70 % Isopropyl Alcohol – 1min

12. 80 % Isopropyl Alcohol – 1min

13. OG_6 solution – 1 min

14. 95 % Isopropyl Alcohol – 1min

15. 95 % Isopropyl Alcohol – 1min

16. EA 50 – 1min

17. 95 % Isopropyl Alcohol – 1min

18. 95 % Isopropyl Alcohol – 1min

19. Absolute Isopropyl Alcohol – 3min
20. Absolute Isopropyl Alcohol – 3min

21. Xylene – 2 min

22. Xylene – 2 min

23. Mount in DPX

Enumeration:

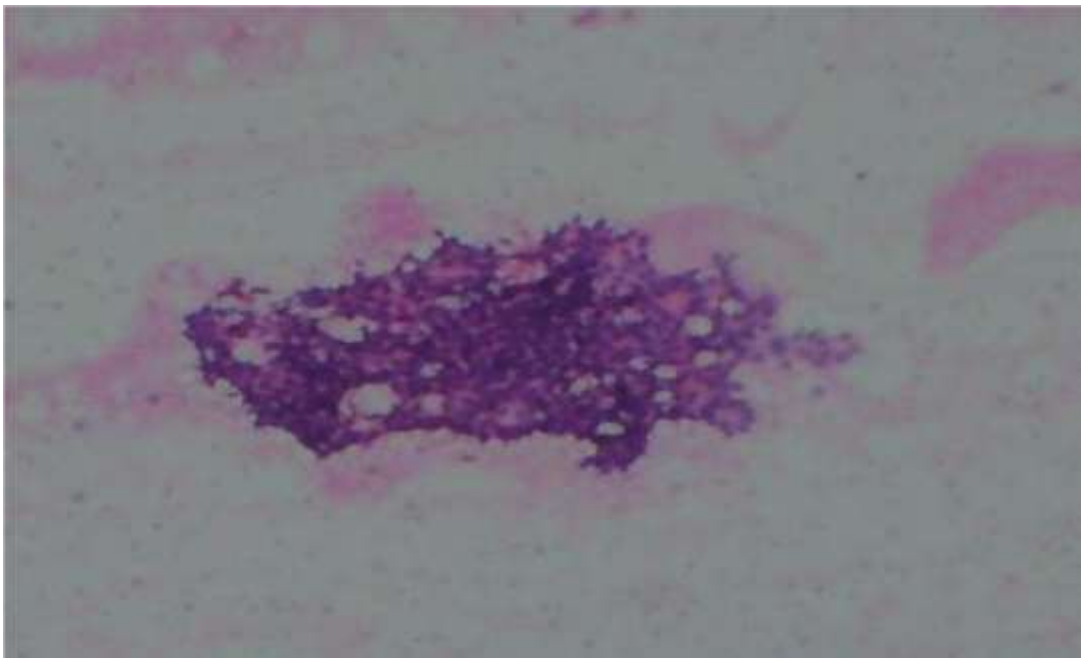
The stained smears were seen under ADelta Plan Z^{TM}AP40 with APCAM-5 Binocular CCD attached microscope.

They are then scored according to the semi quantitative scoring system.

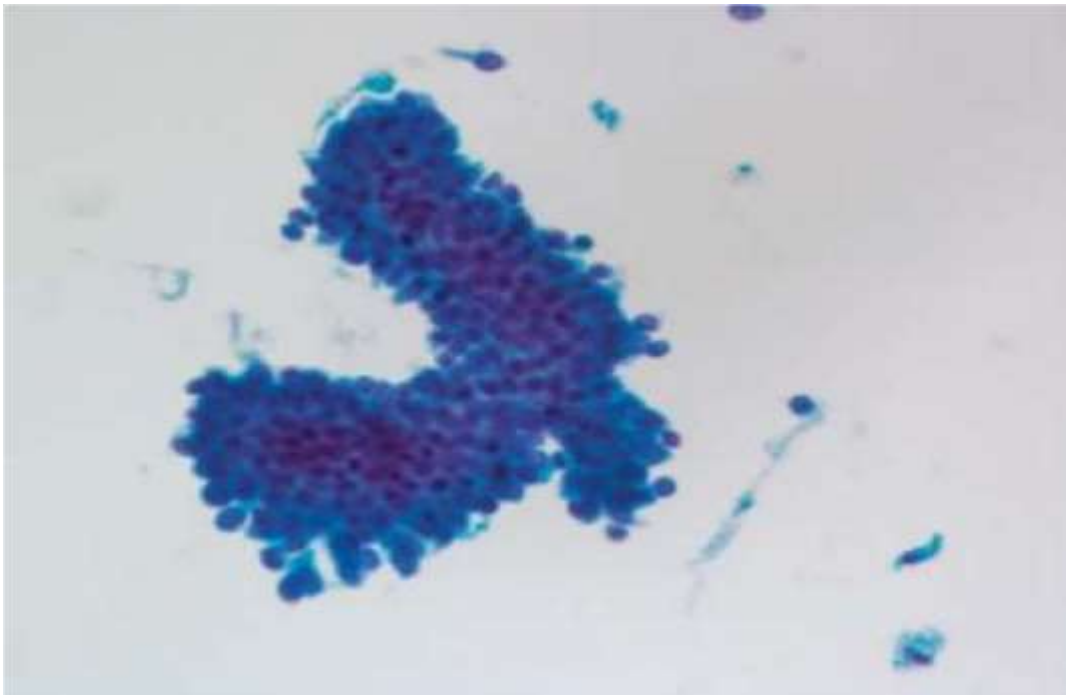
Table 1: Semi quantitative Scoring system used in FNA Smears:				
Cytological features	Score 0	Score 1	Score 2	Score 3
Cellularity	Zero	Scanty	Adequate	Abundant
Background blood & debris	Zero	occasional	Good amount	
Informative background (Colloid, Mucus, and Stromal fragments).	Absent	Present	----- -	----- -
Monolayer	Absent	Occasional	Good amount	------
Cell architecture	Non Recognised	Moderately Recognised	Well Recognised	------
Cytoplasmic details	Poor	Fair	Good	Excellent
Nuclear details	Poor	Fair	Good	Excellent

Statistical Analysis

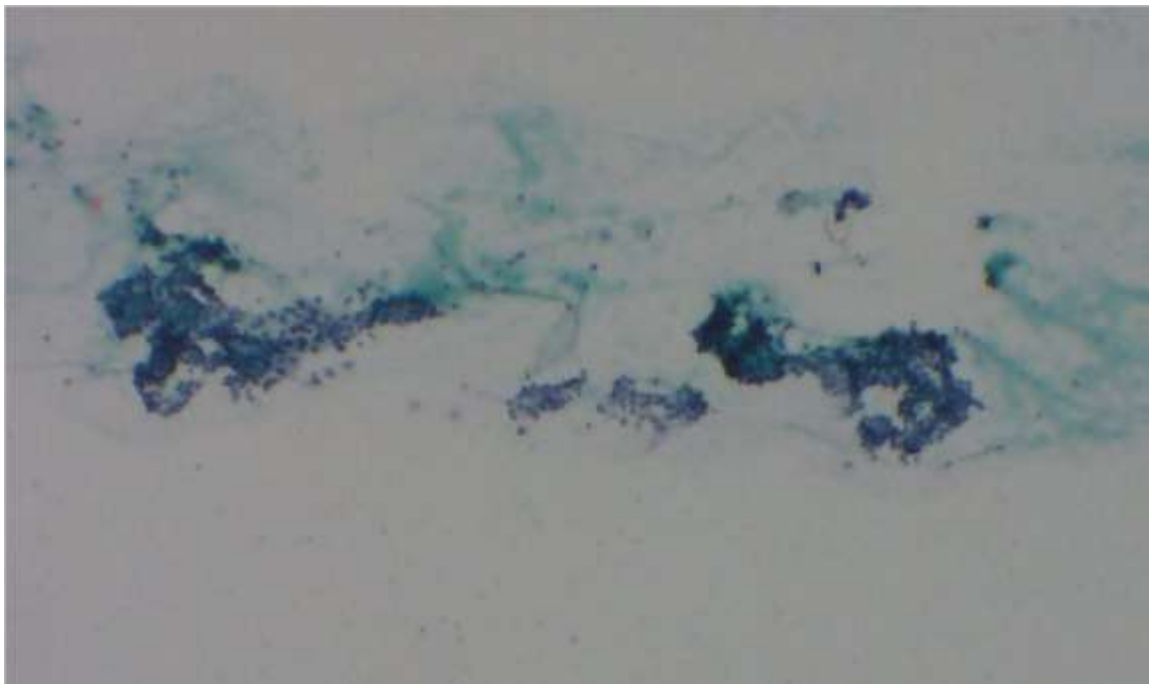
Data collected will be entered in Excel data sheet and analysis done on SPSS software.



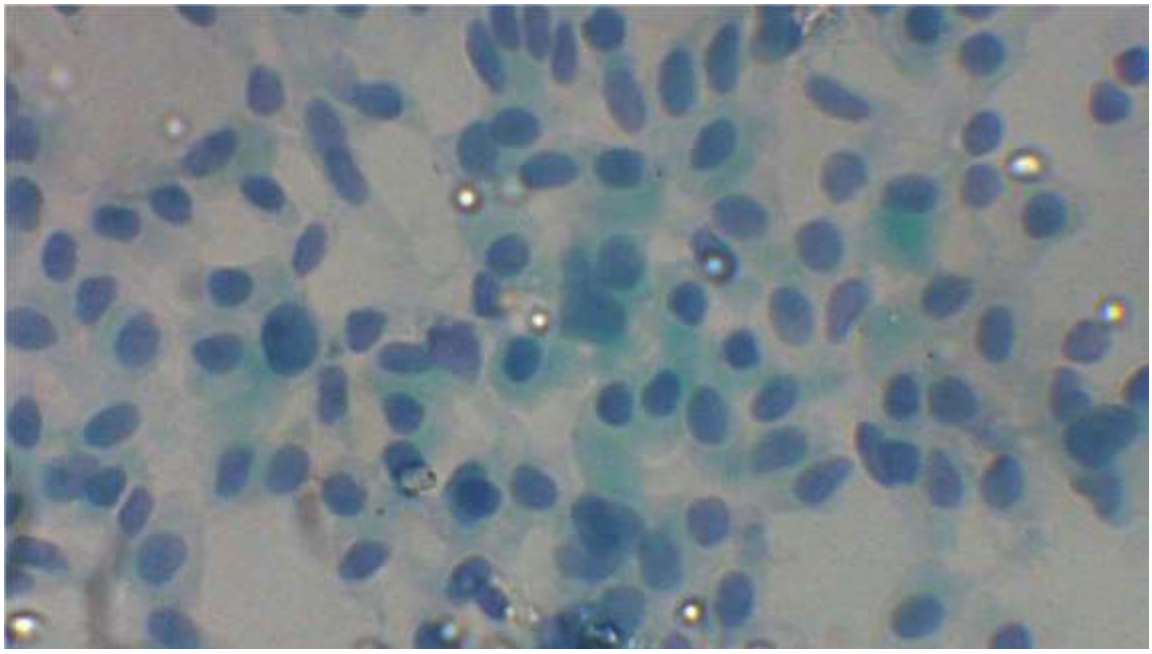
Conventional Smear - Fibroadenoma



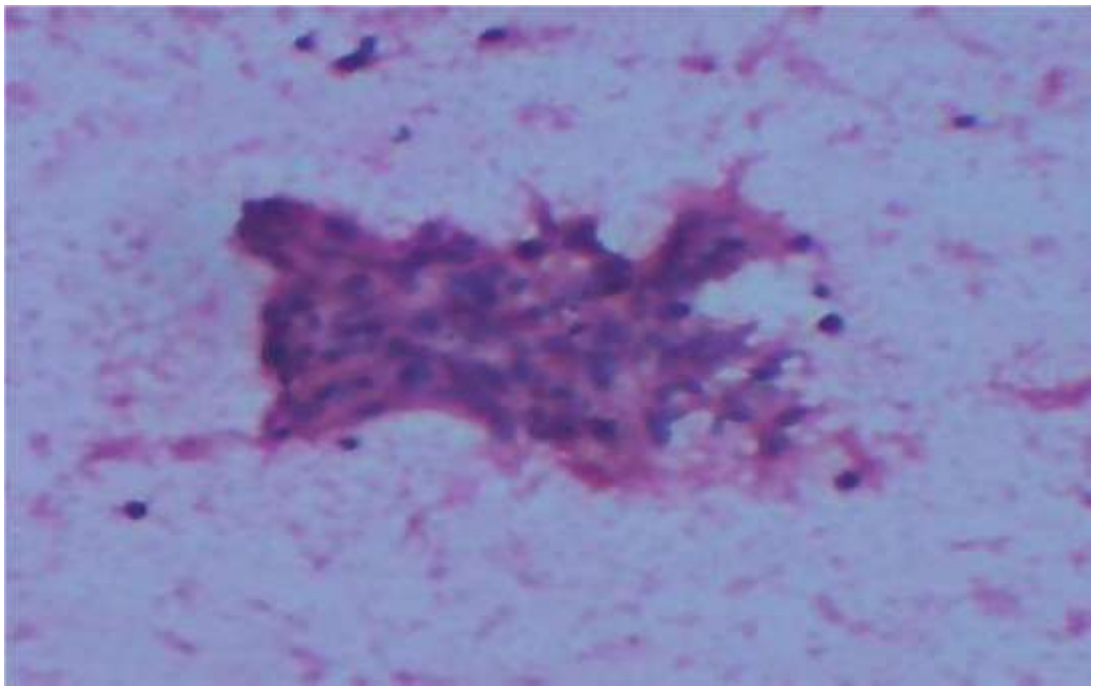
UPREP LBC –Fibroadenoma



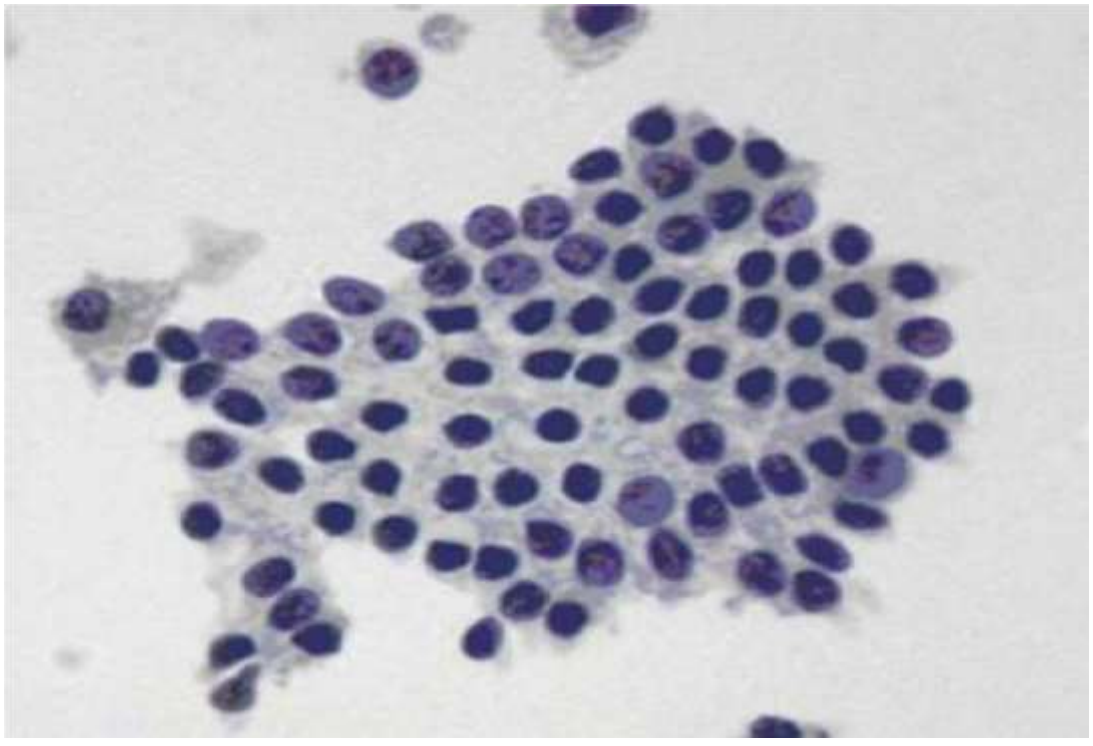
Conventional Smear - carcinoma breast



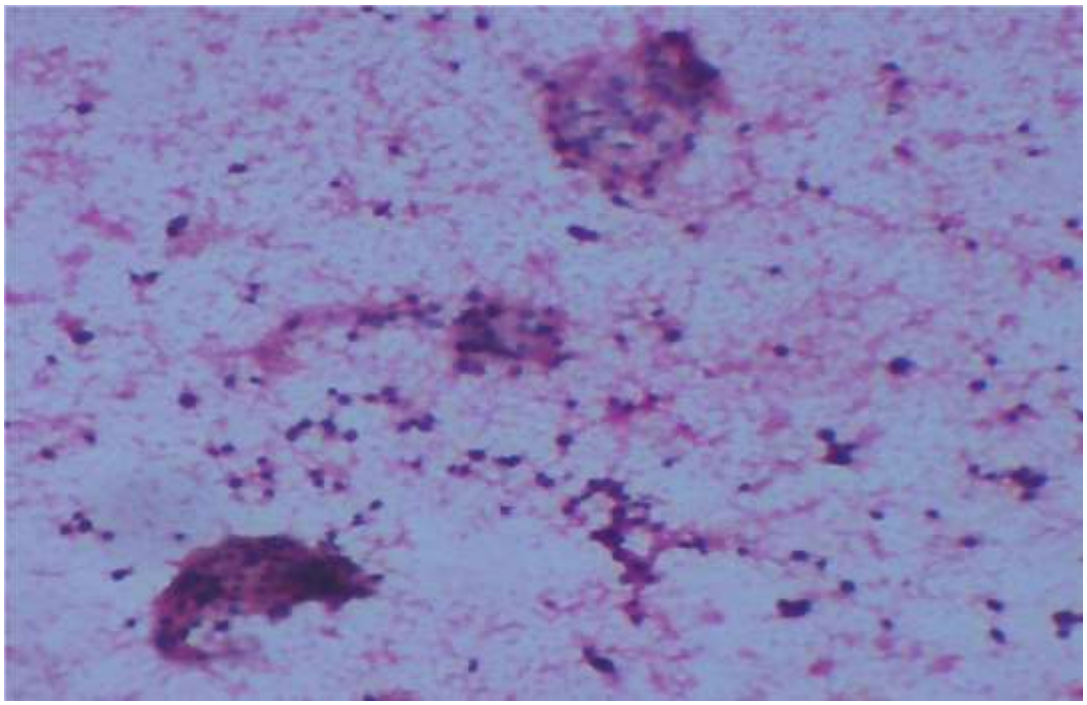
UPREP LBC- Carcinoma Breast



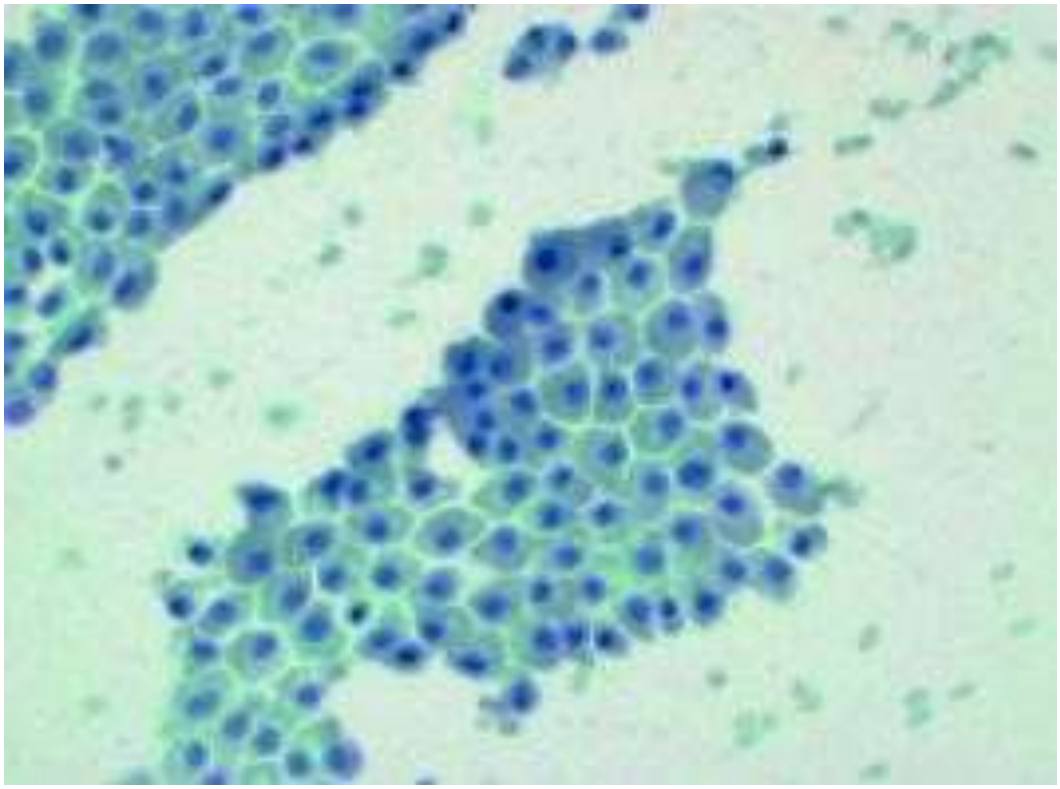
CS – Colloid Goitre



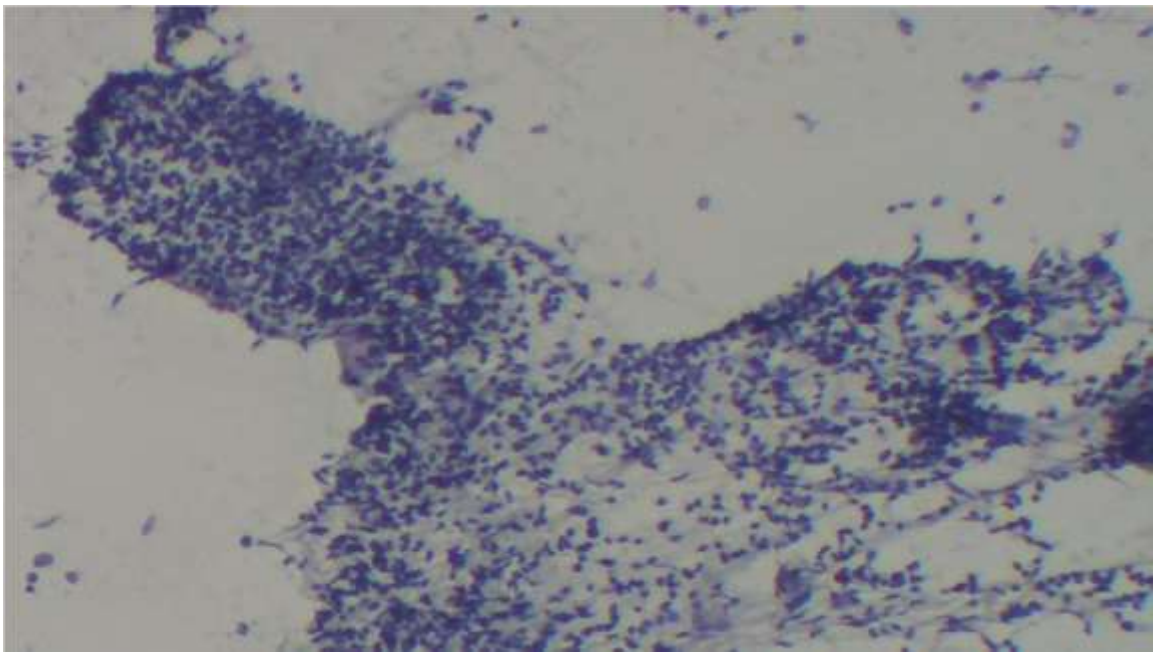
UPREP – Colloid Goitre



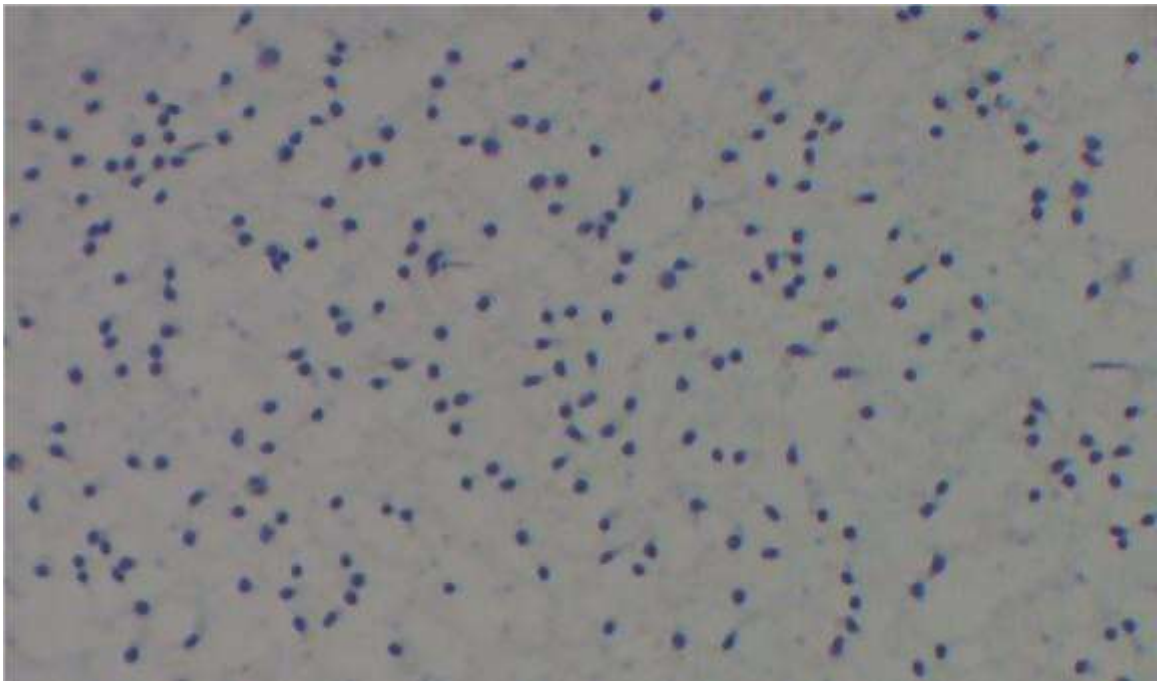
CS- Hashimotos Thyroiditis



UPREP – Hashimotos Throiditis



CS – Reactive lymphadenopathy



UPREP - Reactive lymphadenopathy

STATISTICS

SOCIODEMOGRAPHIC CHARACTERISTICS:

AGE DISTRIBUTION:

The distribution of age in the study participants in both conventional and uprep group ranges from 14 to 67 years. The mean age of study participants in both conventional and uprep groups were 42.78 years (95% CI is 39.523, 46.037) with a SD of 12.327 years.

Distribution according to age of participants:

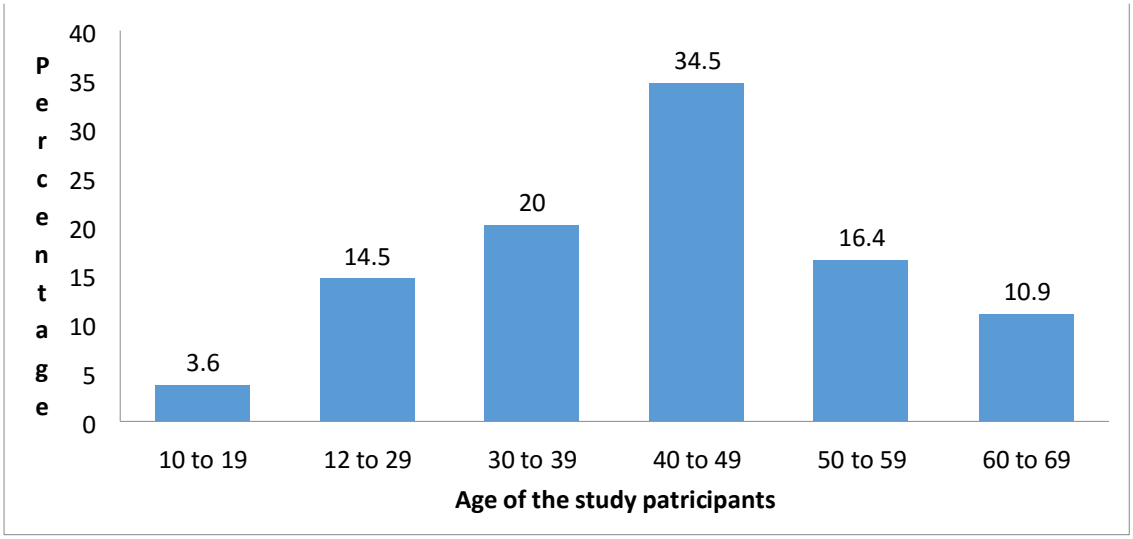
Age characteristics	Conventional group	Uprep group
Minimum	14	14
Maximum	67	67
Mean	42.78	42.78
Standard deviation	12.327	12.327

Majority of the study participants were 40-49 years of age group (34.5%), followed by 30-39 years of age group (20%) in both conventional and uprep groups.

Age distribution in the study population

Age group	Conventional group		Uprep group	
	Frequency	Percent	Frequency	Percent
10-19	2	3.6	2	3.6
20-29	8	14.5	8	14.5
30-39	11	20.0	11	20.0
40-49	19	34.5	19	34.5
50-59	9	16.4	9	16.4
60-69	6	10.9	6	10.9
Total	55	100	55	100

Age of the study participantsin both conventional and uprep groups



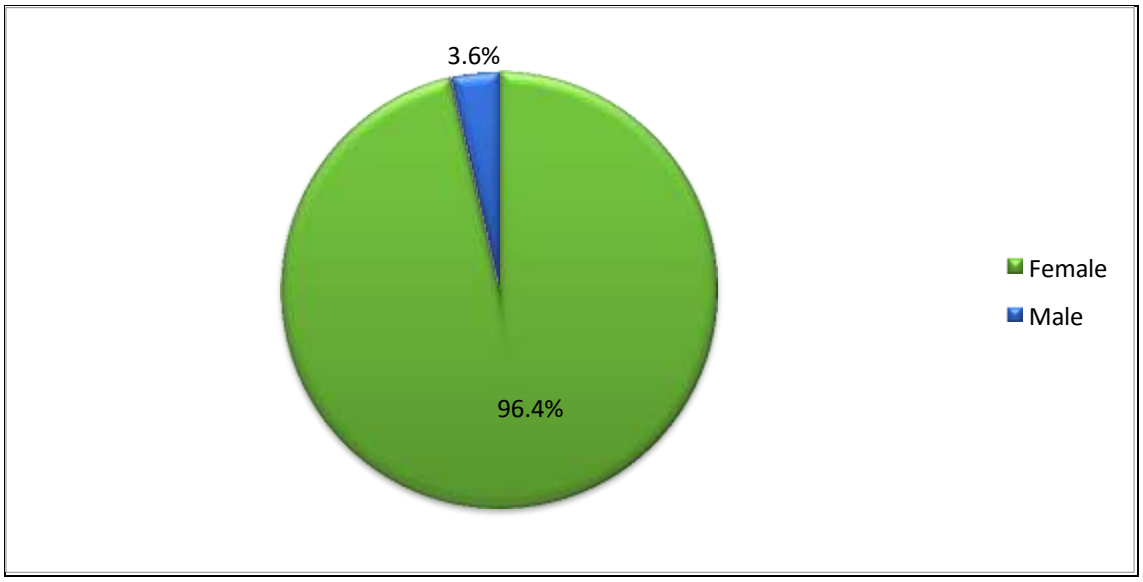
SEX

In this study majority of the study participants were females (96.4%) in both conventional and uprep group.

Distribution of sex in the study population:

Sex	Conventional group		Uprep group	
	Frequency	Percent	Frequency	Percent
Female	53	96.4	53	96.4
Male	2	3.6	2	3.6
Total	55	100	55	100

Gender of the study population in both conventional and uprep groups



SITE

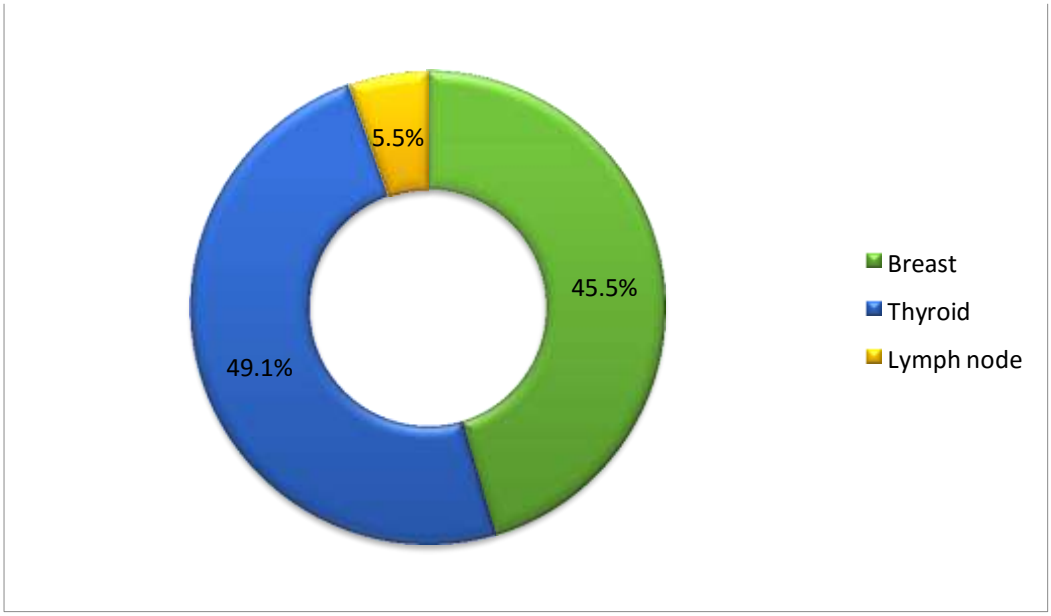
Distribution of site in the study participants

In this study majority of the cases were thyroid lesions (27 cases) followed by breast lesions (25 cases) and lymphadenopathy (3 cases)

Site	Conventional group		Uprep group	
	Frequency	Percent	Frequency	Percent
Breast	25	45.5	25	45.5
Thyroid	27	49.1	27	49.1
Lymph node	3	5.5	3	5.5

Total	55	100	55	100

Site



DIAGNOSIS

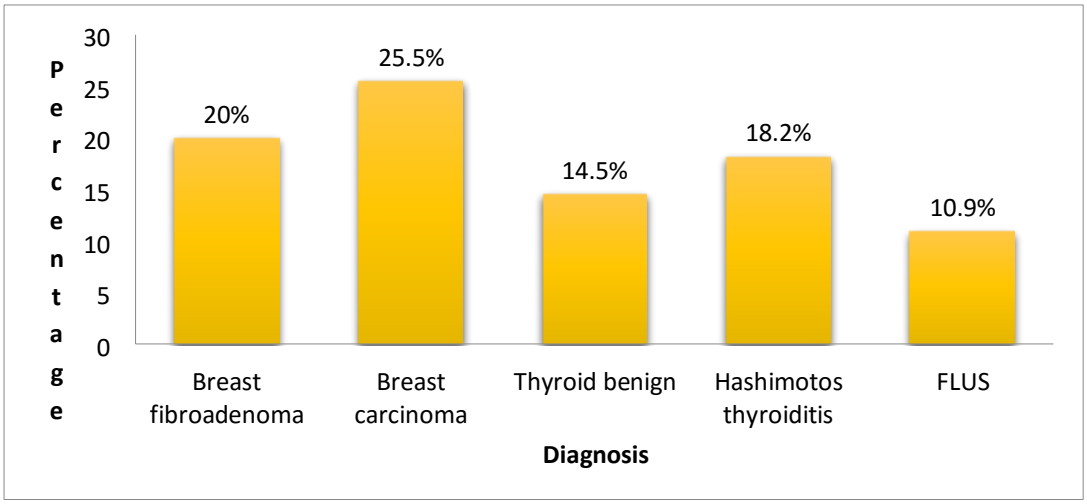
In the present study cytological diagnosis of both groups were similar. Hence the diagnostic accuracy of UPREP LBC was similar to CS with regard to benign and malignant conditions.

Diagnosis in both conventional and uprep groups

Diagnosis	Conventional group		Uprep group	
	Frequency	Percent	Frequency	Percent
	11	20.0	11	20.0

Breast fibroadenoma	14	25.5	14	25.5
Breast carcinoma	8	14.5	8	14.5
Thyroid benign	10	18.2	10	18.2
Hashimotos thyroiditis	6	10.9	6	10.9
FLUS				
Papillary Thyroid Carcinoma	3	5.5	3	5.5
Reactive Lymph node	3	5.5	3	5.5
Total	55	100	55	100

Diagnosis in both conventional and uprep groups



CELLULARITY

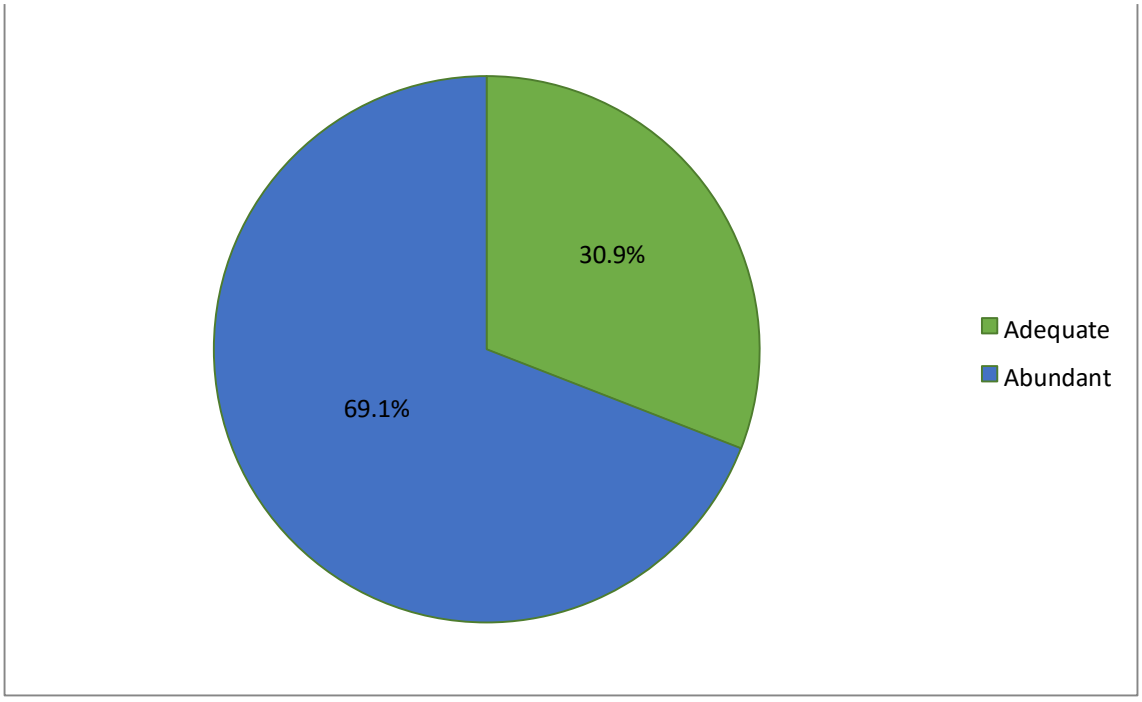
Cellularity in the study population

Cellularity is both the groups are almost similar. CS having an adequate cellularity of 30.9% when compared to 36.4% in MLBC group.

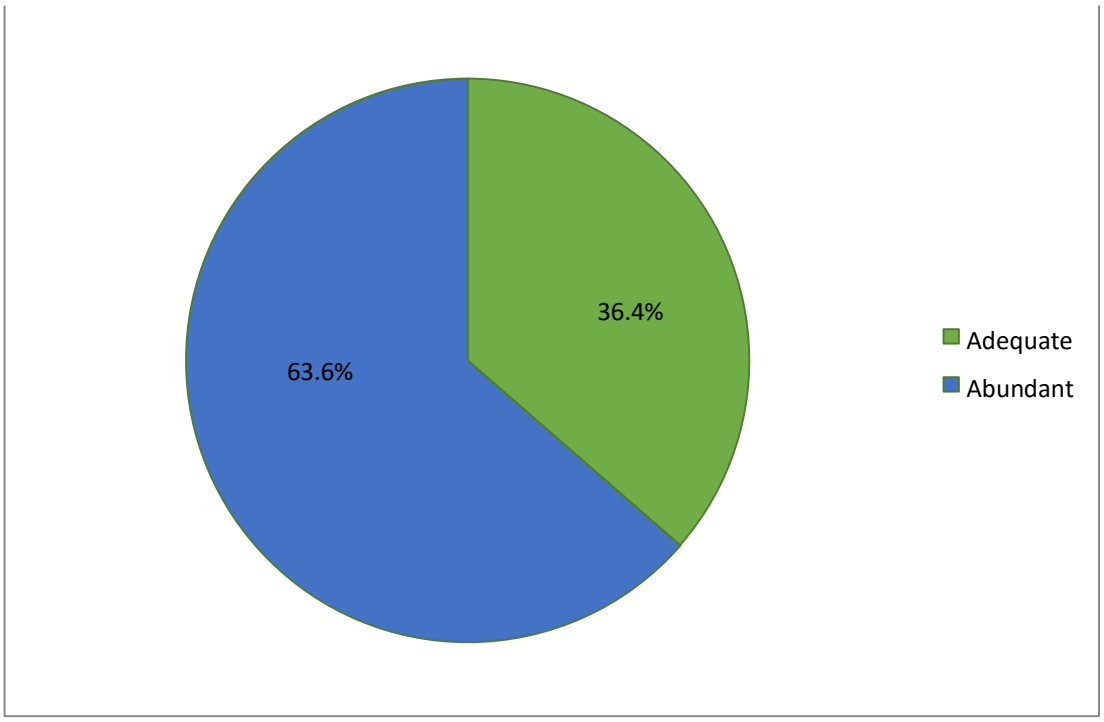
Abundant cellularity was noted in 69.1% in CS group and it was 63.6% in MLBC group.

Cellularity	Conventional group		Uprep group	
	Frequency	Percent	Frequency	Percent
Adequate	17	30.9	20	36.4
Abundant	38	69.1	35	63.6
Total	55	100	55	100

Cellularity in the conventional group



Cellularity in the uprep group

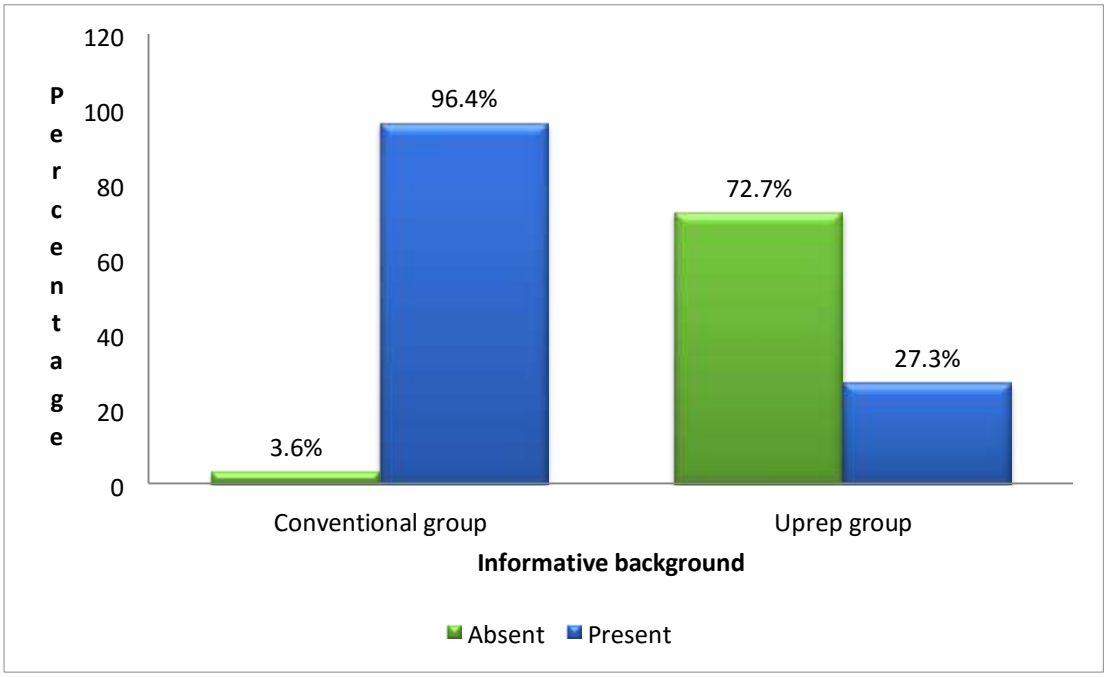


INFORMATIVE BACKGROUND

Informative background in the study population

Informative background	Conventional group		Uprep group	
	Frequency	Percent	Frequency	Percent
Absent	2	3.6	40	72.7
Present	53	96.4	15	27.3
Total	55	100	55	100

Informative background in both conventional and uprep groups

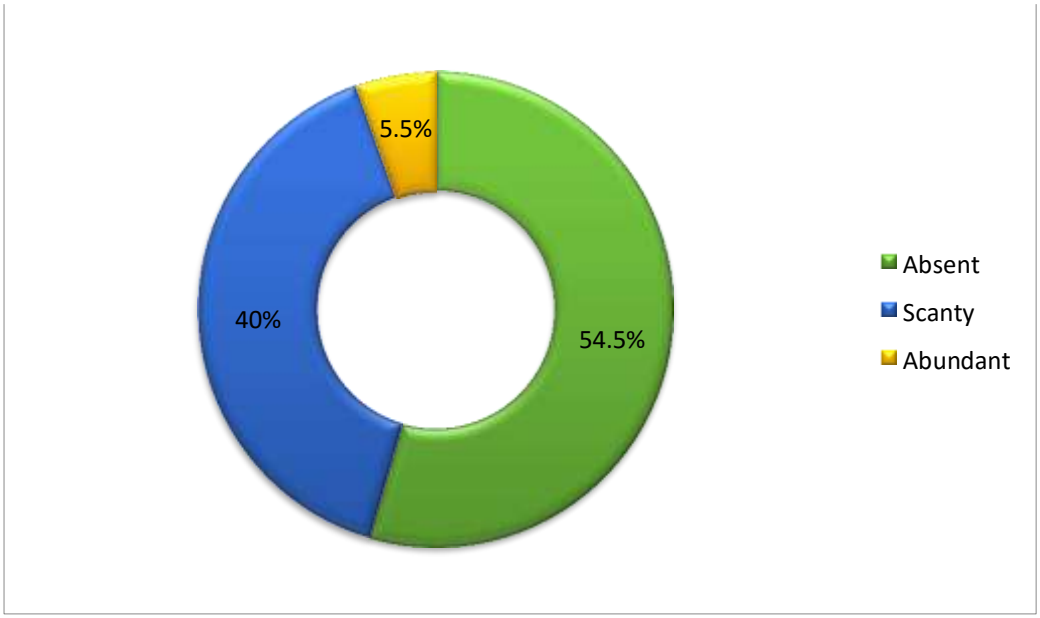


BACKGROUND DEBRIS

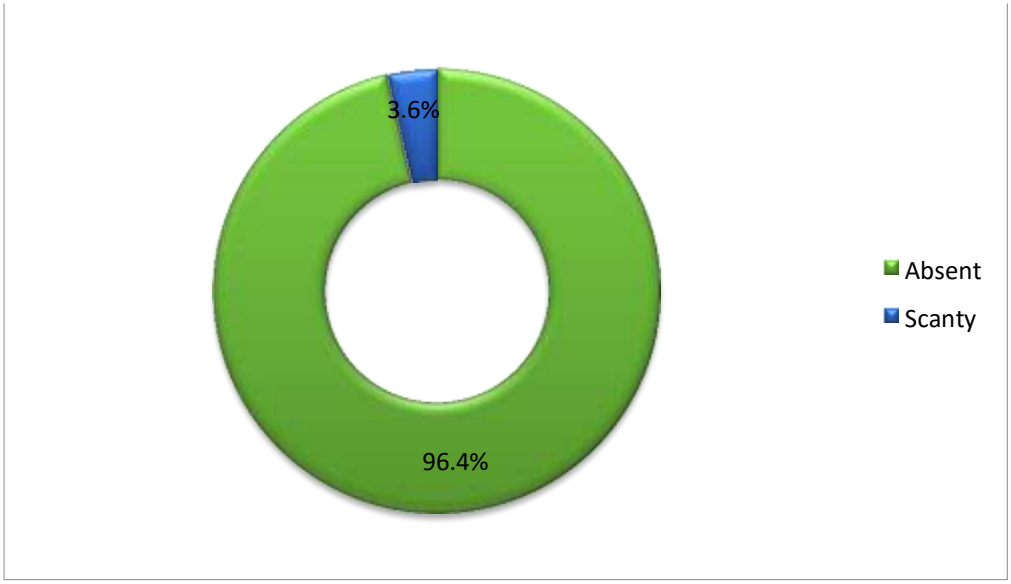
Background debris in both conventional and UPREP groups

Background debris	Conventional group		Uprep group	
	Frequency	Percent	Frequency	Percent
Absent	30	54.5	53	96.4
Scanty	22	40.0	2	3.6
Abundant	3	5.5	0	0
Total	55	100	55	100

Background debris in the conventional group



Background debris in the uprep group



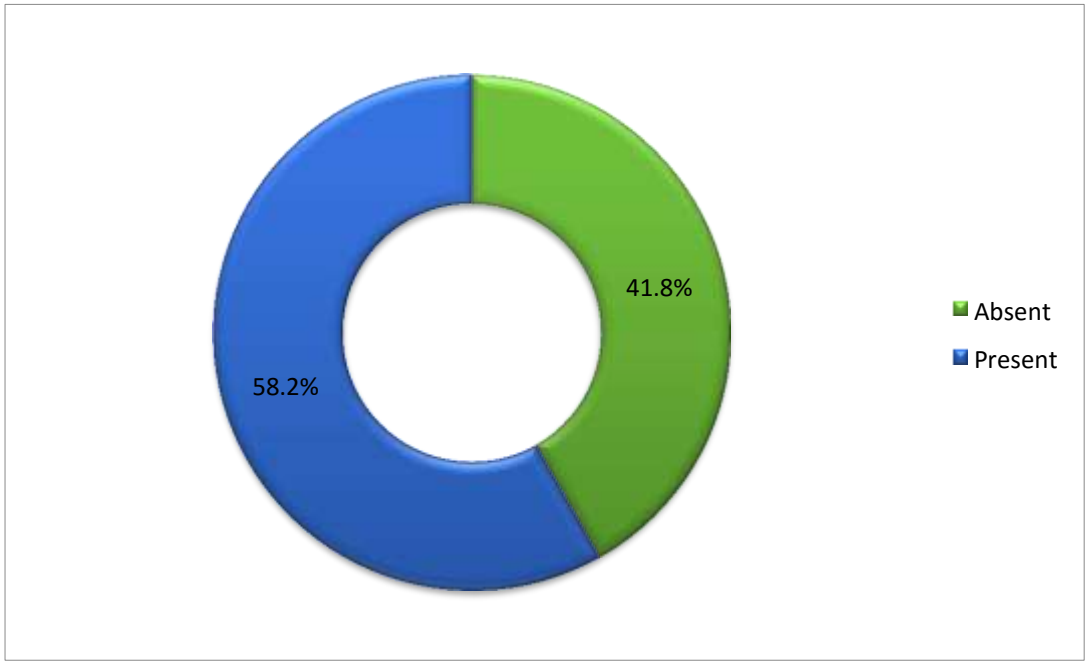
INFLAMMATORY CELLS

Inflammatory cells in the study population

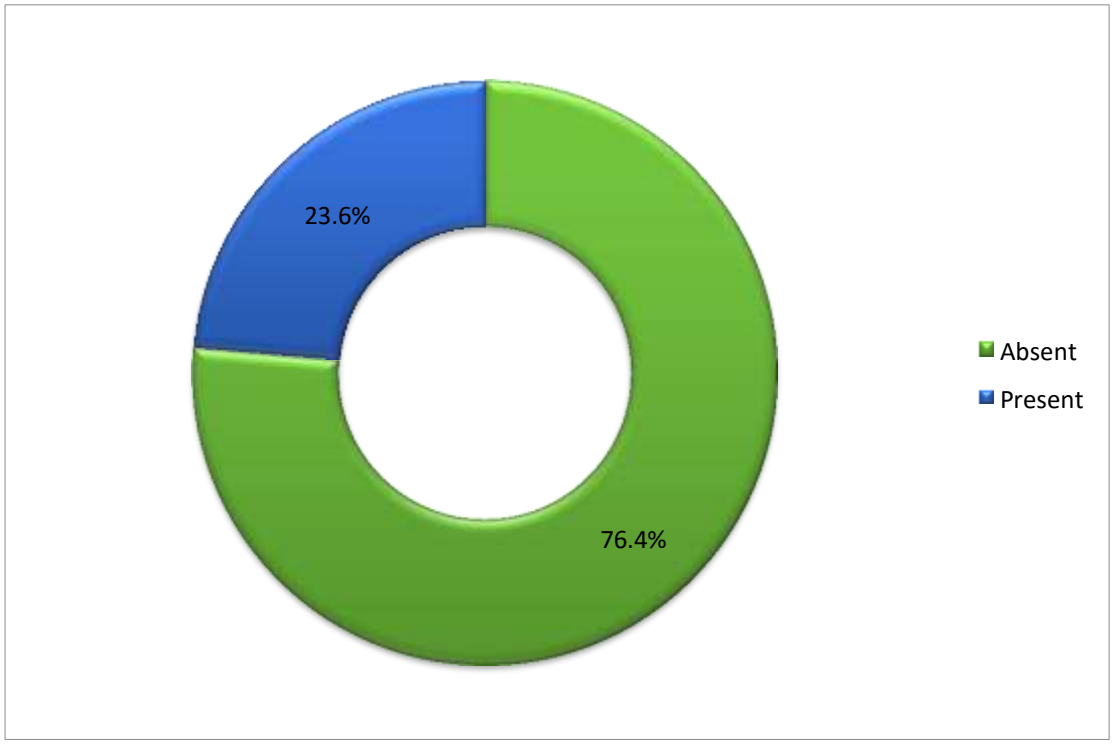
Lymphocytes was the predominant group of inflammatory cells present in the smears studied.

Inflammatory cells	Conventional group		Uprep group	
	Frequency	Percent	Frequency	Percent
Absent	23	41.8	42	76.4
Present	32	58.2	13	23.6
Total	55	100	55	100

Inflammatory cells in the conventional group



Inflammatory cells in the uprep group

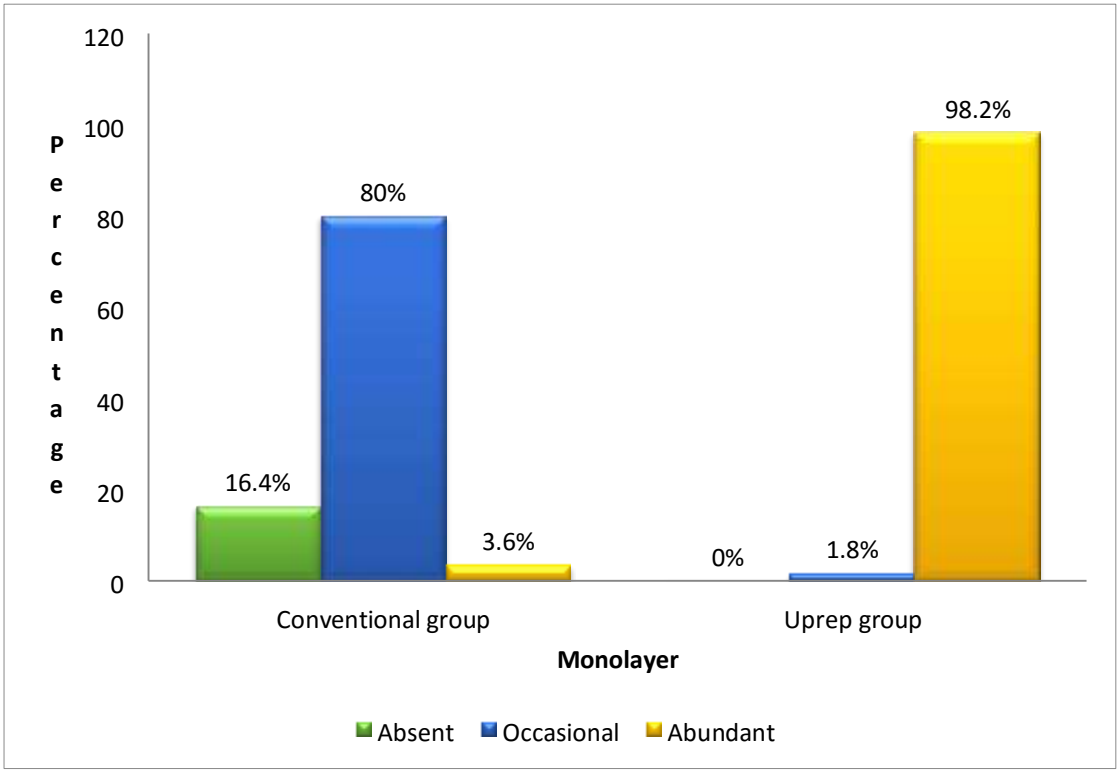


MONOLAYER

Monolayer in the study groups

Monolayer	Conventional group		Uprep group	
	Frequency	Percent	Frequency	Percent
Absent	9	16.4	0	0
Occasional	44	80	1	1.8
Abundant	2	3.6	54	98.2
Total	55	100.0	55	100

Monolayer in both conventional and uprep groups

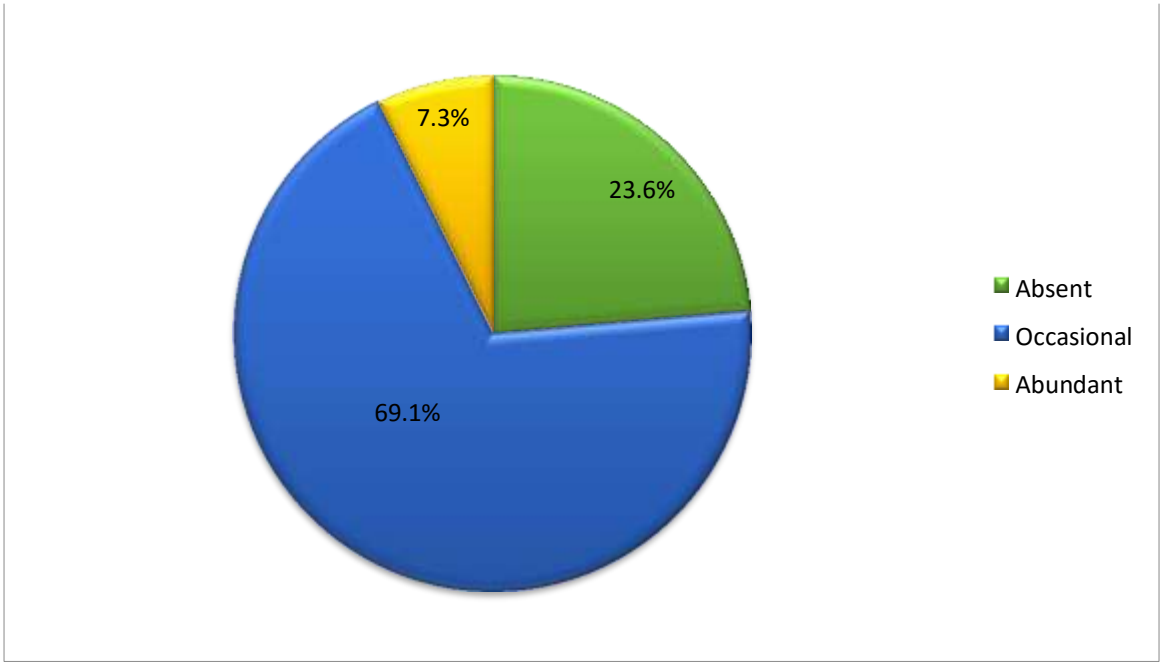


CELL ARCHITECTURE

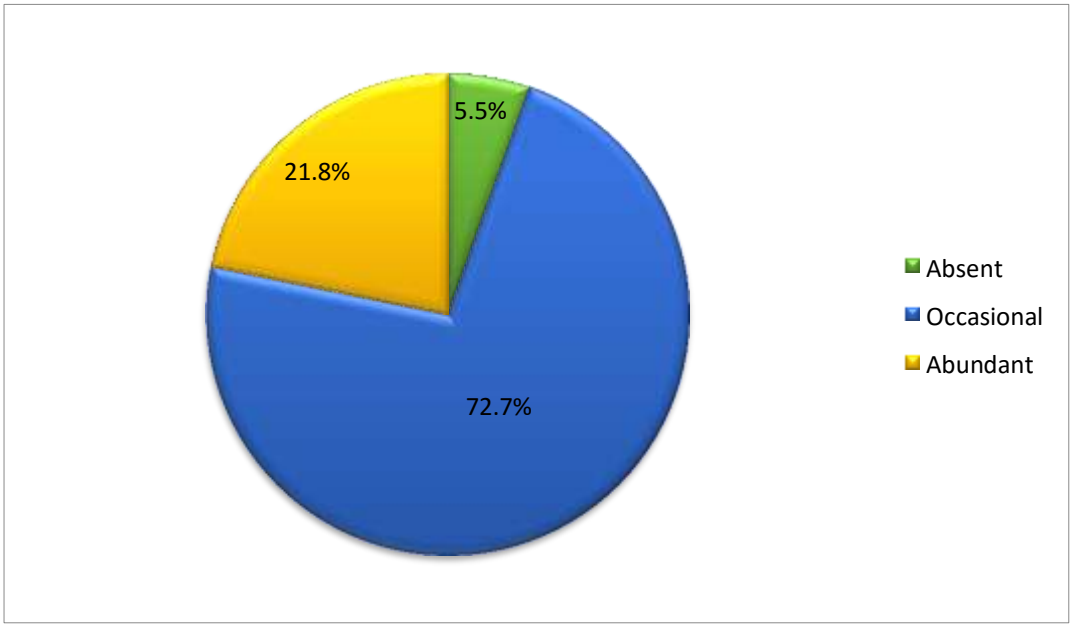
Cell architecture in both conventional and uprep groups

Cell architecture	Conventional group		Uprep group	
	Frequency	Percent	Frequency	Percent
Absent	13	23.6	3	5.5
Occasional	38	69.1	40	72.7
Abundant	4	7.3	12	21.8
Total	55	100	55	100

Cell architecture in the conventional group



Cell architecture in the uprep group

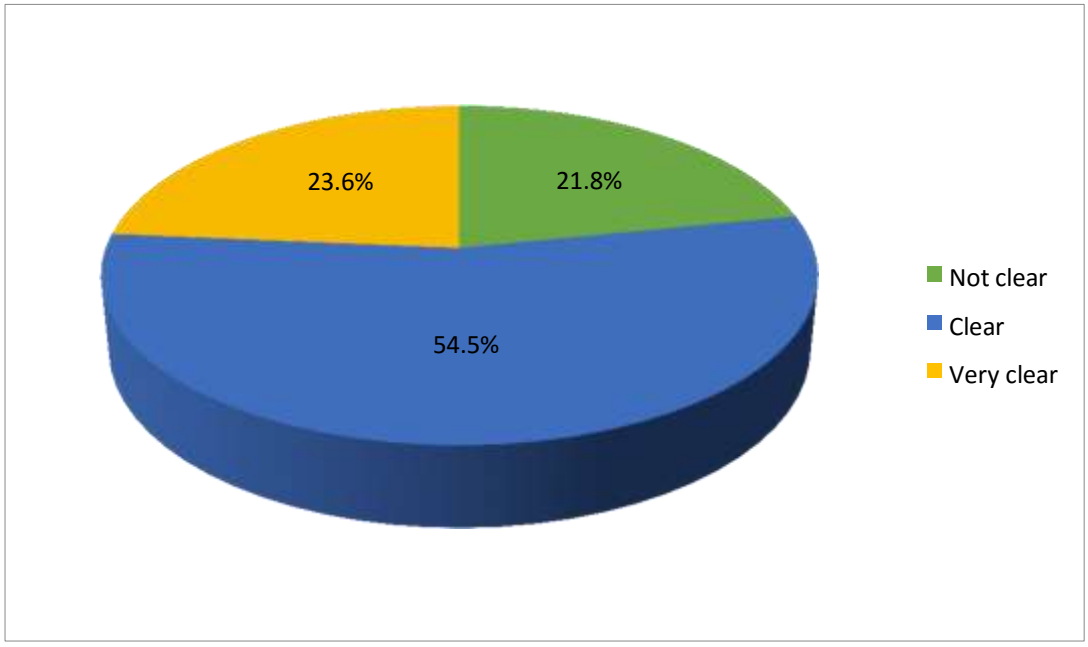


CYTOPLASM

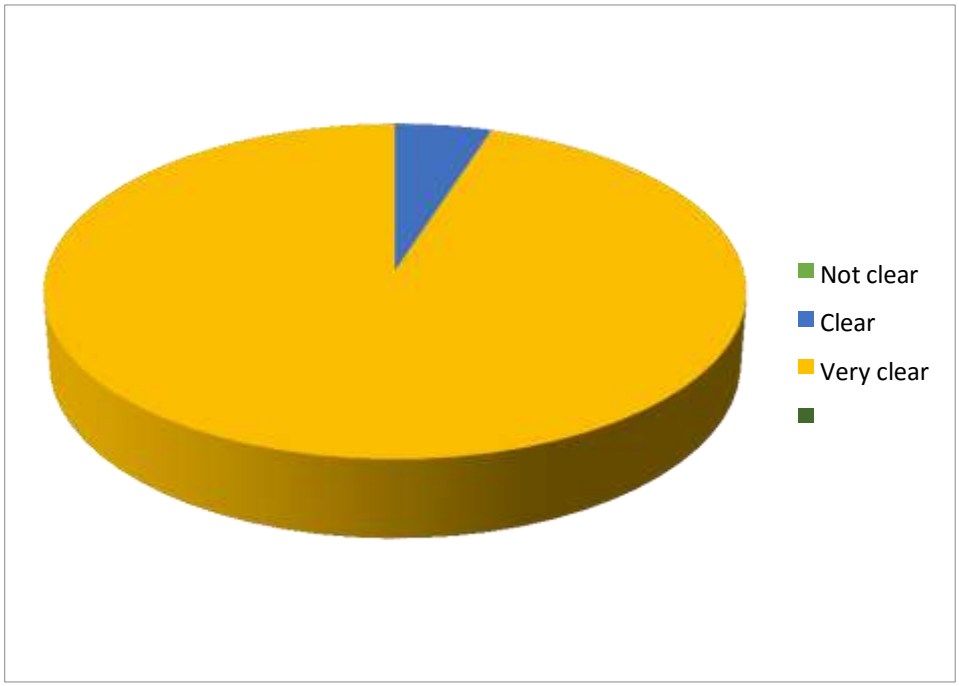
Cytoplasm in both conventional and uprep groups

Cytoplasm	Conventional group		Uprep group	
	Frequency	Percent	Frequency	Percent
Not clear	12	21.8	3	5.5
Clear	30	54.5	0	0
Very clear	13	23.6	52	94.5
Total	55	100	55	100

Cytoplasm in the conventional group



Cytoplasm in the uprep group

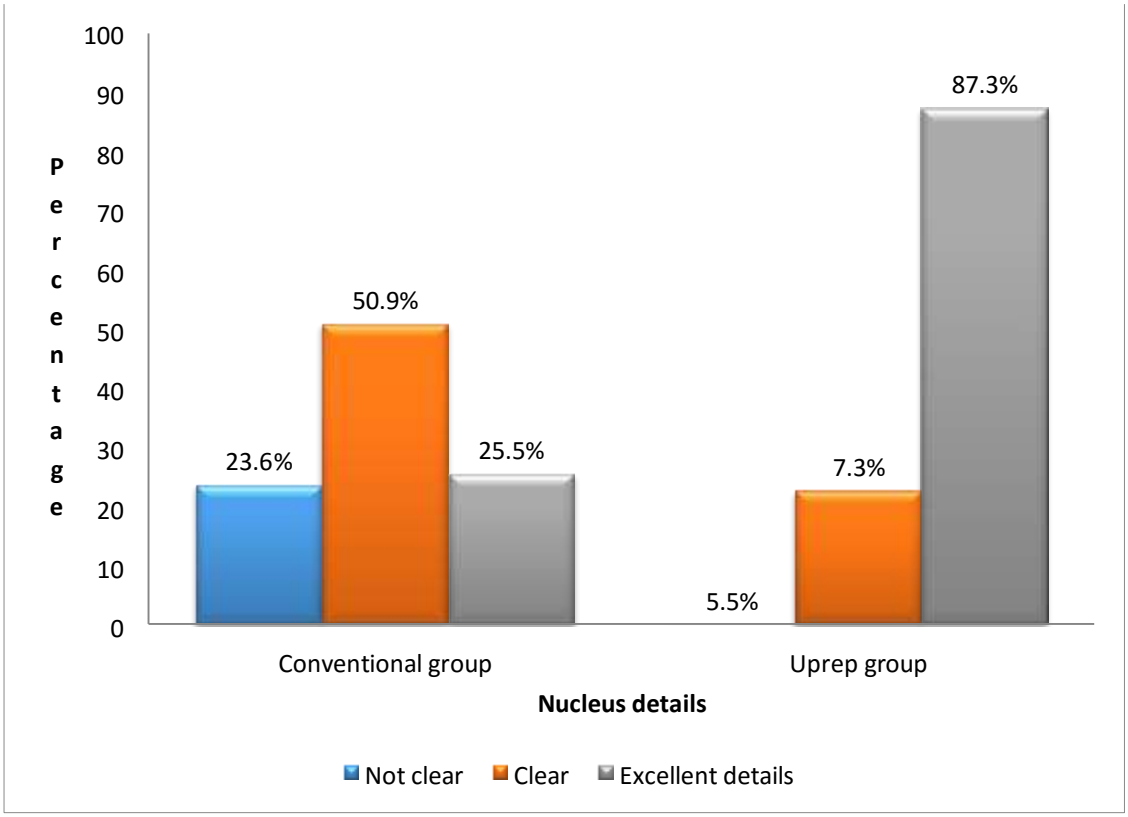


NUCLEUS DETAILS

Nucleus details in the study population

Nucleus details	Conventional group		Uprep group	
	Frequency	Percent	Frequency	Percent
Not clear	13	23.6	3	5.5
Clear	28	50.9	4	7.3
Excellent details	14	25.5	48	87.3
Total	55	100	55	100

Nucleus details in both conventional and uprep groups



RESULT

In the present study comparison of cellularity, informative background, background debris, inflammatory cells, monolayer, cell architecture, cytoplasm and nucleus details of FNAC smears in both conventional and uprep group was done using chi-square test. p value less than 0.05 was considered as significant i.e, there is a difference in FNAC smears made by conventional preparation and UPREP liquid based preparation.

In this study informative background, background debris, inflammatory cells, monolayer, cell architecture, cytoplasm and nucleus details of FNAC smears in both conventional and uprep groups were found to be significant ($p<0.05$).

COMPARISON OF CELLULARITY

Cellularity	Group		Total
	Conventional N (%)	Uprep N (%)	
Adequate	17 (45.9)	20 (54.1)	37
Abundant	38 (52.1)	35 (47.9)	73
Total	55	55	110

$\chi^2 = 0.367$ df = 1 p=0.545

COMPARISON OF INFORMATIVE BACKGROUND

Informative background	Group		Total
	Conventional N (%)	Uprep N (%)	
Absent	2 (4.8)	40 (95.2)	42
Present	53 (77.9)	15 (22.1)	68
Total	55	55	110

$\chi^2 = 55.61$ df = 1 p=0.001*

COMPARISON OF BACKGROUND DEBRIS

	Group	

Background debris	Conventional N (%)	Uprep N (%)	Total
Absent	30(36.1)	53 (63.9)	83
Scanty	22 (91.7)	2 (8.3)	24
Abundant	3 (100)	0	3
Total	55	55	110

$\chi^2 = 27.389$ df = 2 p=0.001*

COMPARISON OF INFLAMMATORY CELLS

Inflammatory cells	Group		Total
	Conventional N (%)	Uprep N (%)	
Absent	23 (35.4)	42 (64.6)	65
Present	32 (71.1)	13 (28.9)	45
Total	55	55	110

$\chi^2 = 13.576$ df = 1 p=0.001

COMPARISON OF MONOLAYER

Monolayer	Group		Total
	Conventional N (%)	Uprep N (%)	
Absent	9 (100)	0	9
Occasional	44 (97.8)	1 (2.2)	45
Abundant	2 (3.6)	54 (96.4)	56
Total	55	55	110

$\chi^2 = 98.375$ df = 2 p=0.001*

COMPARISON OF CELL ARCHITECTURE

Cell architecture	Group		Total
	Conventional N (%)	Uprep N (%)	
Absent	13 (81.2)	3 (18.8)	16
Occasional	38 (48.7)	40 (51.3)	78
Abundant	4 (25)	12 (75)	16
Total	55	55	110

$\chi^2 = 10.301$ df = 2 p=0.006*

COMPARISON OF CYTOPLASMIC DETAILS

Cytoplasm	Gr oup		Total
	Conventional N (%)	Uprep N (%)	
Not clear	12 (80)	0	12
Clear	30 (100)	5	35
Very clear	13 (20)	50 (80)	63
Total	55	55	110

$\chi^2 = 58.8$ df = 2 p=0.001*

COMPARISON OF NUCLEUS DETAILS

Nucleus details	Group		Total
	Conventional N (%)	Uprep N (%)	
Not clear	13 (81.2)	0	13
Clear	28 (87.5)	7 (12.5)	35
Excellent details	14 (22.6)	48 (77.4)	62
Total	55	55	110

$\chi^2 = 42.895$ df = 2 p=0.001* **DISCUSSION:**

Currently, FNA has a significant role in the evaluation of palpable lesions, however the success of FNA depends immensely upon correct preparation of cytological smears and skills of the person performing the procedure. (53-55)

Gerhard R et al observed that the number of passes performed and the skill of the person performing the procedure determine to a large extent the quality and cellularity of the FNA samples. (56)

Liquid based cytology was approved by FDA in 1996 viz. ThinPrep™ (TP; Hologic, Marlborough, Mass., USA) and the SurePath™ (SP; BD TriPath, Burlington, N.C., USA) for gynaecological samples. They helped to overcome problems related to poorly prepared and ill preserved smears. Subsequently the use of LBC extended to non-gynaecological samples including FNA and effusion fluids. (57)

Many authors have evaluated both gynaecological and non-gynaecological specimens using LBC preparations and have attributed benefits over CS namely increased cellularity, lack of obscuring background material, improved morphology, and a decrease in the rate of unsatisfactory or less than optimal specimens.

LBC technique is far easier, quicker, and safer and requires less skill.

The advantages of using the LBC technique are minimal obscuring factors (blood, debris and necrotic materials), excellent cell preservation, lesser fixation artefacts (air-drying), monolayered distribution, less overlapping of the cells and fewer numbers of slides requiring examination.

However, due to chemical influences of the fixation medium and physical force of centrifugation, LBC tends to produce certain cytomorphological alterations and artefacts. Smaller cell clusters and sheets, more dyscohesive cells, breakage of papillae, attenuated chromatin details with prominent nucleoli, intra nuclear inclusion is difficult to visualize, altered background matrix in both quantity and quality, aggregation of lymphocytes and markedly decreased number of extracellular particles, red blood cells, and myoepithelial cells.[58] Hence, interpreting pathologists should be cautious to avoid misinterpretations while reporting FNA prepared using LBC if that is the only methodology employed.[47]

Garbar *et al*. did a study on FNAC of lymph node with CS and LBC at two university hospitals and concluded that despite the cost, the efficiency of lymph node FNAC is identical between CS and LBC. [59]

The present study was conducted in the Department of Pathology, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Tamil Nadu and comprised of 55 cases. Out of which, 11 were fibroadenoma of breast and 14 were carcinoma breast, 8 were benign thyroid lesions, 10 were

hashimotos thyroiditis, 6 were Follicular Lesions of Unknown Significance, 3 were

Papillary Carcinoma Thyroid and two reactive lymphadenopathy.

In each case, conventional smear was prepared from first pass material and MLBC smear was prepared from the second pass material. This was to ensure that the cellularity would not be compromised upon. The diagnosis in both groups were the same hence the diagnostic accuracy of UPREP LBC is similar to conventional smear.

Cellularity

In the present study the cellularity for UPREP LBC was almost equivalent to conventional smear. Dey P. et al in their study utilized a separate pass entirely for MLBC, and the cellularity became almost equivalent to CS.[36] Perez-Reyes et al employed split sampling technique where they divided the aspirate into two halves, one for LBC and the other for CS, hence in their study the cellularity of LBC was inferior to CS.[60] Gerhard R et al observed that the number of passes performed and the skills of the person performing the procedure determine the quality and cellularity of the

samples.[56]

Informative Background

The diagnosis of fibroadenoma breast is classically rendered on the basis of visualization of ductal cell aggregates, bipolar cells and stromal fragments. In the present study, 11 cases of fibroadenoma were analysed. CS met the diagnostic criteria in all cases, however UPREP LBC slides showed an absence of fibromyxoid stroma in all except 1 case. This was in concordance with other studies. Pervez et al concluded that the diagnosis of fibroadenoma seems to be most problematic on LBC preparations.[60] Some studies showed a low diagnostic rate compared to CS and false-positive diagnoses while over classifying fibroadenomas as atypical or suspicious.[40,53,60]

Based on the presence of monolayer, rich cellularity, detailed nuclear features, and clean background, the diagnosis of breast carcinoma was made in the present study. Both LBC and CS preparations have comparable performance for the detection of breast carcinoma. Dey *et al*.,concluded that the diagnosis of ductal carcinoma was easier on LBC due to clean background, lack of necrosis/ haemorrhage and detailed nuclear features of tumor cells.[36]

Amount of colloid in the background plays an important role in the diagnosis of benign and follicular lesions of thyroid. In this study, the amount of colloid on UPREP LBC was diminished and appeared fragmented, and in droplets.

Nuclear grooves and pseudoinclusions were apparent in papillary carcinoma. However, Lee *et al*., observed that background material were slightly superior in LBC preparation than CS preparation. [15] In thyroid lesions, the present study found that MLBC preparations should be interpreted with great caution and CS should always be employed to confirm the diagnosis. Similarly, few

workers demonstrated these problems in their study.[8,61,62]

Background Debris

The amount of obscuring background debris was almost negligible in UPREP LBC preparations when compared to CS preparation. This resulted in better visualization of cells with greater ease of diagnosis and a reduction in the need for repeat FNAC‟s. [44,45,47,50,59,61]

In the current study, there was statistically significant differences between CS preparations and UPREP LBC in view of informative background, background debris, inflammatory cells, monolayer, cell architecture, cytoplasm and nucleus details ($p<0.05$). However, no statistically significant difference was found between these two groups with regard to cellularity, ($P > 0.05$).

CONCLUSION:

Fine needle aspiration is a safe and cost effective method for the diagnosis of palpable lesions at various anatomical sites. However, adequate preparation of smears determines the quality of FNA. Manual liquid based cytology like UPREP LBC is an accurate, less expensive alternative procedure to automated LBC with the advantage of providing monolayer, absence of obscuring blood or debris, better nuclear and cytoplasmic morphology. It is prudent to recognize certain distinct changes in LBC smears in order to avoid interpretative errors. Because of that, training before screening and interpreting LBC preparations is highly recommended. Manual LBC can be used as a adjunct with conventional preparation.

SUMMARY

UPREP liquid based cytology is a novel system of manual liquid based cytology. Automated LBC systems have been in use since the late 1900s. The advantages include improved sensitivity, specificity due to better fixation of cells, reduced air drying artefact, obscuring factors like blood, monolayer preparation, better cytomorphology of nucleus and cytoplasm. This has therefore resulted in a lower rate of unsatisfactory cytology samples. There was also added advantage, the residual material could be used for making further smears, special stains, immunocytochemistry**.** However, the initial high cost of setup have resulted in the development of manual LBC kits.

This study encompassed 55 cases, in whom FNAC was done. Material from the first pass was used to prepare smear in the conventional smear method. Material from the second pass was prepared using UPREP LBC technique. Both slides were compared for cellularity, informative background, background debris, monolayer, cell architecture, nuclear and cytoplasmic details using a semi – quantitative scoring system. The results were analysed using chi-square test.

Out of the 55 cases, 11 were fibroadenoma of breast and 14 were carcinoma breast, 8 were benign thyroid lesions, 10 were Hashimotos Thyroiditis, 6 were Follicular Lesions of Unknown Significance, 3 were

Papillary Carcinoma Thyroid and two were found to be reactive lymphadenitis. The diagnosis in both groups did not change hence the diagnostic accuracy of UPREP LBC is similar to conventional smear. With respect to cellularity no significant difference was noted in both groups. Concerning informative background, UPREP LBC showed less diagnostic background material particularly in benign lesions of breast and thyroid. The absence of necrosis and blood aided in better visualization of cytoplasm and nuclear details particularly in malignant cases.

In the current study, statistically significant differences was observed in CS preparations and UPREP LBC with regard to informative background, background debris, inflammatory cells, monolayer, cell architecture, cytoplasm and nucleus details ($p<0.05$).

Manual liquid based preparations like UPREP LBC can be used as a low cost alternative to other expensive automated LBC systems. Use of both conventional smear and Liquid based cytology can improve the diagnostic yield in fine needle aspiration cytology.

BIBLIOGRAPHY

1. WorldCancerReport2016; http://www.who.int/mediacentre/factsheets/fs297/en. Last accesed on 11.08.2017

2. Derek E Roskell, Ian D Buley.Fine needle aspiration cytology in cancer diagnosis. BMJ. 2004; 329(7460): 244–245.

3. Kavatkar AN, Nagwanshi VA, Dabak S M. Study of a manual method of liquid-based cervical cytology. Indian J PatholMicrobiol 2008; 25(3):190-4.

4. Liquid Based Cytology (LBC), NHS cervical screening programme. Accessed on 20/9/2015.

5. Christian Garber and HervéCuré. Fine-Needle Aspiration Cytology Can Play a Role in Neoadjuvant Chemotherapy in Operable Breast Cancer.

   ISRN Oncology 2013; 20(13):23-35.

6. Material and Methods; www.uprepindia.com; accessed on 26/9/2015

7. Koss Diagnostic cytology and its histologic bases, 5th edition,pg 1570-1670

8. Kalpalata Tripathy, Aparajita Misra, Joydipkumar Ghosh. Efficacy of liquid-based cytology versus conventional smears in FNA samples. J

9. Hutchison et at. Homogeneous sampling accounts for the increased diagnostic accuracy using the ThinPrep Processor. Am J Clin Pathol, 1994 Feb;101(2):215-9.

10. Suvradeep Mitra, Pranab Dey,Fine-needle aspiration and core biopsy in the diagnosis of breast lesions: A comparison and review of the literature, Cytojournal, 2016; 13: 18, PMC5019018

11. Ahmed HG, Ali AS, Almobarak AO, Utility of fine-needle aspiration as a diagnostic technique in breast lumps, Diagn Cytopathol 2009 Dec;37(12):881-4

12. Pandey A., Mishra K.B., Gaur B.S., Singh R.The diagnostic utility of FNAC in palpable lesions of breast at a tertiary care centre, international journal of Medical Research and Review, Vol 5, No 03 (2017)

13. Ashish Kosthi, Maneesh Sulya, Reeni Malik, Role of Fine-Needle Aspiration Cytology in Evaluation of Breast Lumps, Annals of Applied Bio-Sciences, Vol. 4; Issue 3: 2017

14. Scopa, C. D., Koukouras, D., Androulakis, J. and Bonikos, D. (1991), Sources of diagnostic discrepancies in fine- needle aspiration of the breast. Diagn. Cytopathol., 7: 546–548. doi: 10.1002/dc.2840070523

15. Lee Kr, Foster RS, Papillo JL, Fine needle aspiration of the breast. Importance of the aspirator, Acta Cytologica, 01 may 1987, 31(3):281-284, PMID:3473861

16. Katherine T. Morris, Rodney F. Pommier, Arden Morris, Waldemar A. Schmidt, Gregory Beagle, Priscilla W. Alexander, SuEllen Toth-Fejel,

Josh Schmidt, John T. Vetto, Usefulness of the Triple Test Score for Palpable Breast Masses, Arch surg. 2001;136:1008-1013

17. L. A. Brown and S. B. Coghill, “Fine needle aspiration cytology of the breast: factors affecting sensitivity,” Cytopathology, vol. 2, no. 2, pp. 67–74, 1991.

18. F. O'Malley, T. T. Casey, A. C. Winfield, W. H. Rodgers, J. Sawyers, and D. L. Page, “Clinical correlates of false-negative fine needle aspirations of the breast in a consecutive series of 1005 patients,”

19. Scott Boerner M.D.,Nour Sneige M.D.,” Specimen adequacy and false negative diagnosis rate in fine needle aspirats of palpable breast masses”;Cancer Cytopatholgy;1998,volume 84,issue 6;344-348

20. Layfield, L. J., Mooney, E. E., Glasgow, B., Hirschowitz, S. and Coogan, A. (1997), What constitutes an adequate smear in fine-needle aspiration cytology of the breast?. Cancer, 81: 16–21.

Doi:10.1002/(SICI)1097-0142(19970225)81:1<16::AID-CNCR5>3.0.CO;2-E

21. Rabia Basharat, Mulazim Hussain Bukhari, Shahzad saeed, Tahira Hamid; Comparison of Fine Needle Aspiration Cytology and Thyroid Scan in Solitary Thyroid Nodule; Patholog Res Int 2011, 2011:754041

22. Sengupta A, Pal R, Kar S, Zaman FA, Sengupta S, Pal S. Fine needle aspiration cytology as the primary diagnostic tool in thyroid enlargement. Journal of Natural Science, Biology, and Medicine. 2011;2(1):113-118. doi:10.4103/0976-9668.82308.

23. Sukant Garg, Uma Handa, Harsh Mohan, Nitin Nagarkar. Role of fine needle aspiration cytology in diagnosis and management of thyroid lesions: A study on 434 patients.Journal of Cytology, Vol. 25, No. 1, January-March, 2008, pp. 13-17. DOI: 10.4103/0970-9371.40652

24. Hamberger B, Gharib H, Melton LJ III, et al. Fine-needle aspiration biopsy of thyroid nodules: impact on thyroid practice and cost of care. Am J Med. 1982;73:381-384.

25. Yassa L, Cibas ES, Benson CB, et al. Long-term assessment of a multidisciplinary approach to thyroid nodule diagnostic evaluation. Cancer. 2007;111:508-516.

26. Goellner JR, Gharib H, Grant CS, et al. Fine-needle aspiration cytology of the thyroid, 1980 to 1986. Acta Cytol. 1987;31:587-590.

27. Grant CS, Hay ID, Gough IR, et al. Long-term follow-up of patients with benign thyroid fine-needle aspiration cytologic diagnoses. Surgery. 1989;106:980-985.

28. Ravetto C, Colombo L, Dottorini ME. Usefulness of fineneedle aspiration in the diagnosis of thyroid carcinoma: a retrospective study in 37,895 patients. Cancer. 2000;90:357-363.

29. Renshaw AA. Accuracy of thyroid fine-needle aspiration using receiver operator characteristic curves. Am J Clin Pathol. 2001;116:477-482.

30. Yang J, Schnadig V, Logrono R, et al. Fine-needle aspiration of thyroid nodules: a study of 4703 patients with histologic and clinical correlations. Cancer. 2007;111:306-315.

31. McHenry CR, Walfish PG, Rosen IB. Non-diagnostic fine-needle aspiration biopsy: a dilemma in management of nodular thyroid disease. Am Surg. 1993;59:415-419.

32. Gharib H, Goellner JR, Johnson DA. Fine-needle aspiration cytology of the thyroid: a 12-year experience with 11,000 biopsies. Clin Lab Med. 1993;13:699-709.

33. Layfield L, Cochand-Priollet B, LiVolsi V, et al. Post thyroid FNA testing and treatment options: a synopsis of the National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference. Diagn Cytopathol. 2008;36:442-448.

34. Faquin WC, Cibas ES, Renshaw AA. “Atypical” cells in fineneedle aspiration biopsy specimens of benign thyroid cysts. Cancer. 2005;105:71-79.

35. Papillo JL, Lapin D: Cell yield. ThinPrep vs. cytocentrifuge. Acta Cytol.38:33-36 1994 PMID 8291353

36. Dey P. · Luthra U.K. · George J. · Zuhairy F. · George S.S. · Haji B.I. Comparison of ThinPrep and Conventional Preparations on Fine Needle Aspiration Cytology Material. Acta Cytologica 2000;44:46-50 (DOI:10.1159/000326224)

37. Michael CW, Hunter B: Interpretation of fine-needle aspirates processed by the ThinPrep technique. Cytologic artifacts and diagnostic pitfalls. Diagn Cytopathol. 23:6-13 2000 PMID 10907924

38. Michael CW, McConnel J, Pecott J, et al.: Comparison of ThinPrep and TriPath PREP liquid-based preparations in nongynecologic specimens. A pilot study. Diagn Cytopathol. 25:177-184 2001 PMID 11536442

39. Fadda G, Rossi ED: Liquid-based cytology in fine needle aspiration biopsies of the thyroid gland. Acta Cytol. 55:389-400 2011 PMID 21986164

40. Charles v. Biscotti, md, Julie a. Hollow, ct(ascp), Sean m. Toddy, ct(ascp) , a n d kirk a. Easley, ms. Thinprep versus conventional smear cytologic preparations in the analysis of thyroid fine-needle aspiration specimens. A.j.c. am j clin patholp.-august 1995, vol. 104-no. 2:150-153

41. Esther Diana Rossi 1, Gian Franco Zannoni 1, Stefania Moncelsi 1, Egidio Stigliano1, Giuseppe Santeusanio2, Celestino Pio Lombardi 3, Alfredo Pontecorvi 4 and Guido Fadda. Application of liquid-based cytology to fine-needle aspiration biopsies of the thyroid gland.

42. Afify AM, Liu J, Al-Khafaji BM: Cytologic artifacts and pitfalls of fine needle aspirations using ThinPrep. A comparative retrospective review. *Cancer*.93:179-186 2001 PMID11391605

43. Malle D, Valeri RM, Pazaitou-Panajiotou K, et al.: Our experience from the use of ThinPrep technique in thyroid fine needle aspiration. *Acta Cytol*.50:23-27 2006 PMID 16514836

44. Rossi ED, Raffaelli M, Zannoni GF, et al.: Diagnostic efficacy of conventional as compared to liquid-based cytology in thyroid lesions. Evaluation of 10,360 fine needle aspiration cytology cases. *Acta Cytol.* 53:659-666 2009 PMID20014555

45. Jing X, Wey E, Michael CW: Diagnostic value of fine needle aspirates processed by ThinPrep® for the assessment of axillary lymph node status in patients with invasive carcinoma of the breast. *Cytopathology.* 24 (6):372-3762013 PMID23020285

46. Das D. K. (2006). Marginal vacuoles (fire-flare appearance) in fine needle aspiration smears of thyroid lesions: does it represent diffusing out of thyroid hormones at the base of follicular cells? Diagn. Cytopathol. 34, 277–28310.1002/dc.20416

47. Arul P. Utility of manual liquid-based cytology and conventional smears in the evaluation of various fine-needle aspiration samples. J Cytol 2016;33:177-81.

48. Prathima S, Shivarudrappa A, Anil N.S. Diagnostic Efficacy of Manual Liquid Based Cytology in Fine Needle Aspiration Samples. Indian

Journal of Pathology: Research and Practice Volume 6 Number 2, April - June 2017 (Part 2) DOI: http://dx.doi.org/10.21088/ ijprp.2278.148 X.6217.15

49. Bandoh, N., Goto, T., Akahane, T., Ohnuki, N., Yamaguchi, T., Kamada, H., Harabuchi, Y., Tanaka, S. and Nishihara, H. (2016), Diagnostic value of liquid-based cytology with fine needle aspiration specimens for cervical lymphadenopathy. Diagn. Cytopathol., 44: 169–

176. doi:10.1002/dc.23402

50. Shukla, Surabhi & Einstein, A & Shukla, Abhilasha & Mishra, Deepika. (2015). Comparison of specimen adequacy and smear quality in oral smears prepared by manual liquid-based cytology and conventional methods. Journal of Oral and Maxillofacial Pathology. 19.

315. 10.4103/0973-029X.174611.

51. Dr. Karan Mahinderu , Dr. Nandini N, Arnaw Kishore, Dr. Anil Kumar Singh. Manual Liquid Based Cytology in Breast Fine Needle Aspiration – Comparison with the Conventional Smear. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN: 2279-0853, p-

ISSN: 2279-0861.Volume 15, Issue 7 Ver. VII (July. 2016), PP 17-24

52. Suresh Pawar, Prajkta & Uday Gadkari, Rasika & Y Swami, Sunil & R Joshi, Anil. (2014). Comparative study of manual liquid-based cytology (MLBC) technique and direct smear technique (conventional) on fineneedle cytology/fine-needle aspiration. cytology samples. Journal of cytology / Indian Academy of Cytologists. 31. 83-6. 10.4103/09709371.138669.

53. Kocjan G, Bourgain C, Fassina A, Hagmar B, Herbert A, Kapila K, *et al*. The role of breast FNAC in diagnosis and clinical management: a survey of current practice. Cytopathology 2008; 19: 271–8.

54. Simsir A, Rapkiewicz A, Cangiarella J. Current utilization of breast FNA in a cytology practice. Diagn Cytopathol 2009; 37: 140–2.

55. Rosa M, Mohammadi A, Masood S. The value of fine needle aspiration biopsy in the diagnosis and prognostic assessment ofpalpable breast lesions. Diagn Cytopathol 2012; 40: 26–34.

56. Gerhard R, Schmitt FC. Liquid-based cytology in fine needle aspiration of breast lesions: A review. Acta Cytol 2014; 58: 533-42.

57. Cibas ES: Cervical and vaginal cytology; in Cibas ES,Ducatman BS (eds): Cytology: Diagnostic Principles and Clinical Correlates. Philadelphia, Saunders, 2009, pp 1-63.

58. Ren S, Solomides C, Draganova-Tacheva R, Bibbo M. Overview of nongynecological samples prepared with liquid-based cytology medium. Acta Cytol 2014;58:522-32.

59. Garbar C, Remmelink M, Mascaux C. Fine needle aspiration cytology of lymph node: Experience of 2 university hospitals with conventional smears and liquid-based cytology. Acta Cytol 2008;52:418-23.

60. Perez-Reyes N, Mulford DK, Rutkowski MA, Logan-Young W, Dawson AE. Breast fine needle aspiration: a comparison of thinlayer and conventional preparation. Am J Clin Pathol 1994; 102: 349–53.

61. Mygdakos N, Nikolaidou S, Tzilivaki A, Tamiolakis D. Liquid Based Preparation (LBP) cytology versus Conventional Cytology (CS) in FNA samples from breast, thyroid, salivary glands and soft tissues. Our experience in Crete (Greece). Rom J Morphol Embryol 2009;50:245-

50.

62. Köybaşioğlu F, Önal B, Şimşek GG, Yilmazer D, Han Ü. Comparison of ThinPrep and conventional smears in head and neck fine needle aspiration cytology. Turk Patoloji Derg 2008;24:159-65.

63. Orell and Sterrett‟s Fine Needle Aspiration Cytology
64. Fred W. Stewart

65. Kurtycz DFI, Hoerl HD: Thin-layer technology: tempered enthusiasm. Diagn Cytopathol 2000;23:1–5

66. Alves VAF, Bibbo M, Schemitt FCL, Milanezi F, Filho AL. Comparison of manual and automated methods of liquid-based cytology a morphologic study. Acta Cytol 2004; 48: 187-93.

67. Lee, Kelly D, Gravitt PE, FanslerMatosem JA, Clark DP. Validation of a low-cost, liquid based screening method of cervical intraepithelial neoplasia. Am J Obstet and Gynecol 2006; 195: 965-70.

68. Tiwari M. Role of fine needle aspiration cytology in diagnosis of breast lumps. Kathmandu University Medical Journal. 2007 Apr-Jun; 5(2): 215-7

69. The Bethesda System of Reporting Thyroid Cytology,2014, Syed S.Ali, Edmund C. Cibas

70. Thyroid Cytopathology, Sudha R. Kini, 2008 edition

71. Fadda G. · Rossi E. · Mulè A. · Miraglia A. · Vecchio F. · Capelli A.,Diagnostic Efficacy of Immunocytochemistry on Fine Needle

Aspiration Biopsies Processed by Thin-Layer Cytology, Acta Cytologica 2006;50:129-135 (DOI:10.1159/000325920)

72. E. Azavedo, G. Svane, G Auer, Stereotactic Fine Needle Biopsy in 2594 mammographically detected non-palpable lesions,The Lancet volume 333, Issue 8646, 13 may 1989,Pg 1033-1036

73. L. Hann, B S Ducatman, H H Wang, V Fein, J M McIntire, Nonpalpable breat lesions: evaluation by means of fine needle aspiration cytology

74. Frable, W. J. (1984), Needle aspiration of the breast. Cancer, 53: 671– 676. doi:10.1002/1097-0142(19840201)53:3+<671::AID, CNCR2820 531313>3.0.CO;2-A

75. Lucio Palombini, Franco Fulciti, Antonio Vetrani, Gaetano de Rosa, Giuseppe Di Benedetto, Pio Zeppa, Giancarlo Troncone, Fine needle aspiration biopsies of breast masses. A critical analysis od 1956 cases in 8 years, Cancer volume 61, Issue 11, pg 2273-2277.

76. Hall, T. L., Layfield, L. J., Philippe, A. and Rosenthal, D. L. (1989), Sources of diagnostic error in fine needle aspiration of the thyroid. Cancer, 63: 718–725. doi:10.1002/1097-0142(19890215) 63:4<718:: AID-CNCR2820630420>3.0.CO;2-N

77. Lioe, T. F., Elliott, H., Allen, D. C. and Spence, R. A. J. (1999), The role of fine needle aspiration cytology (FNAC) in the investigation of superficial lymphadenopathy; uses and limitations of the technique. Cytopathology, 10: 291–297. doi:10.1046/j.1365-2303.1999.00183.x

78. Salhadar, A., Massarini-Wafai, R. and Wojcik, E. M. (2001), Routine use of ThinPrep® method in fine-needle aspiration material as an adjunct to standard smears. Diagn. Cytopathol., 25: 101–103. doi:10.1002/dc.2012

79. Luu, M. H., Fischer, A. H., Pisharodi, L. and Owens, C. L. (2011), Improved preoperative definitive diagnosis of papillary thyroid carcinoma in FNAs prepared with both ThinPrep and conventional smears compared with FNAs prepared with ThinPrep alone. Cancer Cytopathology, 119: 68–73. doi:10.1002/cncy.20124

80. Zajdela, A., Zillhardt, P. and Voillemot, N. (1987), Cytological diagnosis by fine needle sampling without aspiration. Cancer, 59: 1201–1205. doi:10.1002/1097-0142(19870315)59:6<1201::AID-CNCR2820590628>3.0.CO;2-P

ABBREVATIONS

HPV	-	Human Papilloma Virus
FNAC	-	Fine Needle Aspiration Cytology
FDA	–	Food and Drug Agency
CP	–	Conventional Preparation
TP	–	ThinPrep
LBC	–	Liquid Based Cytology
MLBC	–	Manual Liquid Based Cytology
ND/US	-	Non diagnostic or Unsatisfactory
HT	–	Hashimotos Thyroiditis
AUS	–	Atypia of Undetermined Significance
FLUS	–	Follicular Lesion Of Undetermined Significance
PTC	–	Papillary Thyroid Carcinoma

ANNEXURE

The Bethesda System for Reporting Thyroid Cytology (TBSRTC):

For a thyroid FNA specimen to be satisfactory for evaluation (and benign), at least 6 groups of benign follicular cells are required. Each group should be composed of at least 10 cells. The minimum size requirement for the groups allows one to determine (by the evenness of the nuclear spacing) whether they represent fragments of macro follicles. [25,26] Categories :

I. Non diagnostic or Unsatisfactory:

1. Cyst fluid only.

2. Virtually acellular specimen.

3. Other (obscuring blood, clotting artefact).

II. Benign:

1. Consistent with a benign follicular nodule (includes

adenomatoid nodule, colloid nodule, etc).

2. Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context Consistent with granulomatous (sub-

acute) thyroiditis.

3. Other.

III. Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance.

IV. Follicular Neoplasm or Suspicious for a Follicular Neoplasm (Specify if Hürthle cell (oncocytic) type). V.

Suspicious for Malignancy:

1. Suspicious for papillary carcinoma

2. Suspicious for medullary carcinoma

3. Suspicious for metastatic carcinoma

4. Suspicious for lymphoma

5. Other

VI. Malignant:

1. Papillary thyroid carcinoma.

2. Poorly differentiated carcinoma.

3. Medullary thyroid carcinoma.

4. Undifferentiated (anaplastic) carcinoma.

5. Squamous cell carcinoma.

6. Carcinoma with mixed features (specify).

7. Metastatic carcinoma.

8. Non-Hodgkin lymphoma.

9. Others.

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES
KULASEKHARAM – 629 161
Case Record Sheet

STUDY TITLE : "CONVENTIONAL PREPARATION VERSUS UPREP-LIQUID BASED PREPARATION IN FINE NEEDLE ASPIRATION IN A TEACHING HOSPITAL – A COMPARATIVE STUDY"

DATE :

NAME

AGE/SEX

LAND LINE/MOBILE NO :

ADDRESS :

PRESENTING COMPLAINTS :

PAST HISTORY :

OTHERS :

INVESTIGATIONS :

FINDING BY MICROSCOPY :

Parameters	CP	UPREP – LBC
a) Cellularity		
b) Adequacy		
c) Background		
d) Cytoplasmic changes		
e) Nuclear changes		
f) Inflammatory infiltrate:		

INFERENCE:

Dr. Aswathy Jayachandran

CONSENT FORM

PART – 2 of 2

PARTICIPANTS CONSENT FORM

The details of the study have been explained to me in writing and the details have been fully explained to me. I am aware that the results of the study may not be directly beneficial to me but will help in the advancement of medical sciences. I confirm that I have understood the study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the study titled "CONVENTIONAL PREPARATION VERSUS UPREP- LIQUID BASED PREPARATION IN FINE NEEDLE ASPIRATION IN A

TEACHING HOSPITAL – A COMPARATIVE STUDY"

Serial no/Reference no :

Name :

Address :

Contact no :

Signature of the participant

Witness

1.

2.

Date:

Place: Kulasekharam

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES

(Kulasekharam (K.K District, TN)-629161, Phone No: 04651-280866, Fax No: 280740)

Institutional Human Ethics Committee (IHEC)

{CDSCO Reg No: ECR/446/Inst/TN/2013}

Ref. No: SMIMS/IHEC/2015/A/11 **Date: 17th February 2016**

CERTIFICATE

This is to certify that the Research Protocol Ref. No. SMIMS/IHEC/2015/A/11 entitled "Conventional Preparation Versus Uprep-Liquid Based Preparation in Fine Needle Aspiration in a Teaching Hospital-A Comparative Study," submitted by Dr. Aswathy Jayachandran, Postgraduate of Department of Pathology, SMIMS has been approved by the Institutional Human Ethics Committee at its meeting held on 10th December 2015.

17.2.16.

Dr. Rema Menon. N
Member Secretary
Institutional Human Ethics Committee
Professor and HOD of Pharmacology
SMIMS, Kulasekharam (K.K District)
Tamil Nadu-629161

[This Institutional Human Ethics Committee is organized and is operating according to the requirements of ICH-GCP/GLP guidelines and requirements of the Amended Schedule-Y of Drugs and Cosmetics Act, 1940 and Rules 1945 of Government of India.]

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES

KULASEKHARAM

RESEARCH COMMITTEE

CERTIFICATE

This is to certify that The Research Protocol Submitted by Dr ASWATHY JAYACHANDRAN

~~*Faculty*~~ */ Post Graduate from Department of* PATHOLOGY

Titled CONVENTIONAL PREPARATION VERSUS UPKEP LIQUID BASED PREPARATION IN FINE NEEDLE ASPIRATION IN A TEACHING HOSPITAL – A COMPARITIVE STUDY

is approved by the Research Committee.

Chair Person
Prof & HOD
Dept. of Bio Chemistry
Sree Mookambika Institute of Medical Sciences
Kulasekharam 629 161

Convenor
Prof. & H.O.D.
Dept. of Physiology
Sree Mookambika Institute of Medical Sciences
Kulasekharam 629 161

Date :

AGE	SEX	SITE	DIAGNOSIS	CS- CELLULARITY	LBC CELLUI	CS INFORM	LBC INFOR	CS BACKGR	LBCBACKG	CS INFLAM	LBC INFLAI	CS MONOL	LBC MONC	CS CELL AR	LBC CELL A	CS CYTOPL	LBC CYTOP	CS NUCLEA	LBC NUCLEAR DETAILS
52	1	1	2	2	2	1	0	1	0	1	0	1	2	2	2	2	0	2	0
34	1	2	5	2	2	0	0	1	1	0	0	1	1	2	1	2	1	2	1
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60	1	1	2	3	2	1	0	1	0	1	0	1	2	2	2	3	3	3	2
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54	1	3	7	3	3	1	0	0	0	0	0	0	2	2	2	3	3	3	3
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55	1	1	2	3	2	1	0	2	0	1	0	1	2	3	2	3	3	3	3
66	1	2	4	3	3	1	0	0	0	1	0	1	2	2	2	2	3	2	3
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42	1	2	3	2	2	1	1	0	0	1	0	1	2	2	2	2	3	2	3
29	1	1	1	3	3	1	1	0	0	0	0	1	2	1	2	2	3	2	3
50	1	2	3	3	3	1	0	1	0	1	0	1	2	1	3	1	3	1	3
48	1	1	2	3	3	1	0	1	0	1	0	1	2	1	2	1	3	1	3
53	1	2	4	2	2	1	0	0	0	1	0	2	2	1	3	2	3	2	3
55	1	2	5	3	3	1	0	0	0	0	0	1	2	2	3	2	3	2	3
45	1	2	4	3	2	1	0	0	0	1	0	1	2	1	2	1	3	1	3
44	1	3	7	3	3	1	0	1	0	1	1	1	2	1	3	1	3	1	3
41	1	2	5	3	3	1	0	0	0	1	1	1	2	2	3	1	3	1	3
38	1	1	1	3	3	1	0	0	0	0	0	1	2	2	3	1	3	1	3
45	1	2	6	3	3	1	0	1	0	1	0	0	2	1	2	0	3	0	3
37	1	2	5	2	2	1	0	1	0	0	0	1	2	2	2	2	3	2	3
26	1	2	4	2	2	1	1	0	0	1	1	1	2	2	2	2	3	2	3
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49	1	1	1	2	2	1	0	0	0	1	0	1	2	2	2	2	3	2	3
45	1	1	2	3	3	1	0	1	0	1	1	1	2	1	2	1	3	1	3
30	1	1	1	2	2	1	1	1	0	1	0	1	2	1	2	1	3	1	3
46	1	2	4	3	3	1	1	0	0	1	1	1	2	2	2	2	3	2	3

AGE	SEX	SITE	DIAGNOSIS	CS- CELLULARITY	LBC CELLUI	CS INFORM	LBC INFORI	CS BACKGR	LBCBACKG	CS INFLAM	LBC INFLAI	CS MONOL	LBC MONC	CS CELL AR	LBC CELL A	CS CYTOPL	LBC CYTOP	CS NUCLEA	LBC NUCLEAR DETAILS
35	1	1	1	2	2	1	0	0	0	0	0	1	2	2	2	2	3	2	3
22	1	1	1	3	3	1	0	0	0	0	0	1	2	2	2	2	3	2	3
28	1	2	6	3	3	0	0	0	0	0	0	1	2	2	1	2	3	2	3
45	1	1	1	3	3	1	0	0	0	0	0	1	2	2	2	2	3	2	3
67	1	1	2	3	3	1	0	1	0	0	0	1	2	2	2	2	3	2	3
38	1	1	2	3	3	1	0	1	0	1	0	1	2	2	2	2	3	2	3
47	1	1	2	3	3	1	0	1	0	1	1	1	2	2	2	2	3	2	3
26	1	2	5	3	3	1	0	0	0	0	0	1	2	1	2	2	3	1	3
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35	1	1	1	2	2	1	0	0	0	0	0	2	2	2	2	2	3	2	3
60	1	1	2	3	3	1	0	1	0	1	1	1	2	2	2	2	3	2	3

FEMALE-1
MALE-2

BREAST -1
THYROID-2
LYMPH NODE-3

BREAST FIBROADENOMA-1
BREAST CARCINOMA-2
THYROID BENIGN-3
THYROID HASHIMOTO THYROIDITIS-4
THYROID FLUS-5
THYROID CARCINOMA-6
LYMPH NODE 7